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(54) Title: HYBRID AND CHIMERIC POLYPEPTIDES THAT REGULATE ACTIVATION OF COMPLEMENT

(57) Abstract: A hybrid complement-regulating protein comprises a first functional unit of a first complement regulatory protein having complement regulating properties, a first spacer sequence of at least about 200 amino acids encoding a polypeptide that does not exhibit complement regulating properties and at least a second functional unit attached to the spacer sequence. The second functional unit may be a polypeptide providing a functional unit of a second complement regulatory protein, a polypeptide derived from an immunoglobulin, or a polypeptide that enhances binding of the protein to an animal cell. The hybrid protein may also contain a second spacer sequence and a third functional unit of a complement regulatory protein, a polypeptide derived from an immunoglobulin, and a polypeptide that enhances binding of the protein to an animal cell. The optional third functional unit may be the same or different from the first or second functional units.

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INTERNATIONAL SEARCH REPORT

International application No.

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A. CLASSIFICATION OF SUBJECT MATTER

IPC(8): A61K 38/16(2006.01);C07K 14/00(2006.01),14/705(2006.01)

USPC: 530/350,380,387.3,402,403;514/2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 530/350, 380, 387.3, 402, 403; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FODOR, W.L. et al. A novel bifunctional chimeric complement inhibitor that regulates C3 convertase and formation of the membrane attack complex. J. Immunol. 1 November 1995, Vol. 155, No. 9, pages 4135-4138, see entire document.	1-11, 14, 15

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

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INTERNATIONAL SEARCH REPORT

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 12 and 13
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Claim 12 recites sequence identifiers, however no sequence listing has been provided. Claim 13 is dependent upon the polypeptide of claim 11, but claim 11 is drawn to a nucleic acid vector.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
 2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of any additional fees.
 3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
 4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
- Remark on Protest
- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
 - ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
 - ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

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Continuation of B. FIELDS SEARCHED Item 3:
EAST, Medline, Embase

TERMS: DAF, CD55, CR1, CD35, IgG4, fusion, chimeric, hybrid

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**HYBRID AND CHIMERIC POLYPEPTIDES THAT REGULATE ACTIVATION OF
COMPLEMENT**

**STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR
DEVELOPMENT**

[0001] The present invention was made, at least in part, with support from NIH grant #AI23598. The U.S. government may have certain rights in this invention.

BACKGROUND OF THE INVENTION

[0002] The complement system comprises a number of serum proteins that function in the body's immune response to infection and tissue injury. Activation of complement can occur via three pathways, the classical pathway involving the binding of complement component C1q to antigen-antibody complexes, the lectin pathway involving binding of mannose binding lectins to antigens, and the alternative pathway involving binding of complement component C3b to an activator surface such as cell wall polysaccharides of yeast and bacterial microorganisms. Activation of complement results in the formation of anaphylatoxins (C3a and C5a), membrane attack complexes (C5b-9), and opsonins (C3b and C4b) that amplify inflammation and destroy foreign and necrotic cells.

[0003] Complement activation is regulated by a number of plasma and cell associated proteins. Such proteins inactivate specific steps of the classical, lectin, and/or alternative pathway by regulating the activity of C3/C5 convertases or serving as a cofactor for the factor I cleavage of C3b and/or C4b. These proteins are either soluble plasma proteins or membrane proteins (integral or lipid-anchored) expressed on a variety of cell types. These proteins possess many structural similarities.

[0004] Decay Accelerating Factor (DAF)

[0005] Decay accelerating factor (DAF, CD55) is a membrane-associated regulatory protein that protects self cells from activation of autologous complement on their surfaces. DAF acts by rapidly dissociating C3 and C5 convertases, the central enzymes of the cascade. DAF

possesses the most potent decay accelerating activity of the proteins associated with complement regulation, and acts on both the classical pathway (C4b2a and C4b2a3b) and alternative pathway (C3bBb and C3bBbC3b) enzymes. DAF, however, does not have cofactor function.

[0006] Structural analyses of DAF have shown that, starting from its N-terminus, it is composed of four ~60 amino acid-long units followed by a heavily O-glycosylated serine (S) and threonine (T) rich stretch, which is, in turn, linked to a posttranslationally-added glycoinositolphospholipid (GPI) anchor. The amino acid sequence of DAF is shown in Fig 1 A (SEQ. ID NO: 1). The four 60 amino acid long repeating units are termed complement control protein repeats (CCPs) or short consensus repeats (SCRs). CCP1 includes amino acids 35-95 of SEQ. ID NO: 1. CCP2 includes amino acids 97-159; CCP3 includes amino acids 162-221 and CCP4 includes amino acids 224-284 of SEQ. ID NO: 1. They provide for all of DAF's regulatory activity. The heavily O-glycosylated region serves as a cushion which positions the CCPs at an appropriate distance above the surface membrane. The GPI anchor allows DAF to move freely in the plane of the plasma membrane enabling it to inactivate convertase complexes wherever they assemble.

[0007] The critical role that DAF plays in inhibiting complement activation is evident both from natural disease and studies in animal models employing *Daf* knockout mice. In the human disease paroxysmal nocturnal hemoglobinuria (PNH), mutation in the GPI anchor pathway leading to the absence of DAF renders affected blood cells susceptible to heightened C3b uptake and intravascular hemolysis. In the animal disease models employing the *Daf* knockout, the absence of DAF renders the mice markedly more susceptible to tissue damage in 1) nephrotoxic serum (NTS) induced nephritis, a model of human membranous glomerulonephritis, 2) dextran sodium sulfate (DSS) induced colitis, a model of inflammatory bowel disease, and 3) anti-acetylcholine receptor (anti-AChR) induced myasthenia gravis, a close model of the human autoimmune disorder.

[0008] The nucleotide sequence of a cDNA encoding DAF is shown in Fig. 1B (SEQ. ID NO: 2).

[0009] Complement Receptor 1 (CR1)

[0010] Complement receptor 1 (CR1 or the C3b receptor, CD35) is another potent regulator of complement activation. Unlike DAF which functions *intrinsically* to protect the cells that express it, CR1 functions *extrinsically* on targets of complement attack, e.g. pathogens. CR1 is a larger molecule in that, rather than 4 CCPs, it is comprised of 30 CCPs arranged in 4 groups of 7 CCPs termed long homologous repeats (LHRs). The CCPs and LHRs of CR1 are provided in Table I below. The amino acid residue numbers refer to the amino acid sequence provided in Fig 2 (SEQ. ID NO: 3). Functional analyses have shown that CR1 possesses both decay accelerating activity and cofactor activity for cleavage of C4b and C3b by the serum enzyme, factor I. Early studies showed that among complement regulators, it is the most potent in this latter activity and that it is the only regulator that promotes both initial cleavage of C3b to iC3b and subsequent cleavage of the iC3b intermediate to C3dg, the surface-bound C3b end product.

[0011] Structure-function studies of CR1 have shown that its regulatory activity resides primarily in its three N-terminal LHRs, i.e., LHRs A, B, and C. Functional activity within each 7 CCP LHR is contained essentially in each case in the first 3 CCPs. Recent studies have shown that CR1's potent cofactor activity resides in LHRs B and C, while its decay accelerating activity resides in LHR A.

[0012] The nucleotide sequence of a cDNA encoding CR1 is shown in Fig 3 (SEQ. ID NO: 4).

Table 1

Amino Acid No.	Domain
1 or 6-46	Leader peptide
47-106	CCP1, begin LHR-A
107-168	CCP2
169-238	CCP3
239-300	CCP4
301-360	CCP4
361-423	CCP5
424-496	CCP7, end LHR-A
497-556	CCP8, begin LHR-B
557-618	CCP9
619-688	CCP10
689-750	CCP11
751-810	CCP12
811-873	CCP12
874-946	CCP14, end LHR-B
947-1006	CCP15, begin LHR-C
1007-1068	CCP15
1069-1138	CCP16
1139-1200	CCP17
1201-1260	CCP18
1261-1323	CCP20
1324-1399	CCP21, end LHR-C
1400-1459	CCP22, begin LHR-D
1460-1521	CCP23
1522-1591	CCP24
1592-1653	CCP25
1654-1713	CCP26
1714-1776	CCP27
1777-1851	CCP28, and LHR-D
1852-1911	CCP29
1912-1972	CCP30

[0013] Membrane Cofactor Protein (MCP)

[0014] MCP (also known as 'CD46') is present on the cell surface of a number of cell types including peripheral blood cells (excluding erythrocytes), cells of epithelial, endothelial and fibroblast lineages, trophoblasts and sperm. MCP has four CCPs and a serine/threonine enriched region in which heavy O-linked glycosylation occurs. MCP also has a transmembrane and cytoplasmic domain. The structure of MCP is provided in Table 2 below with reference to the amino acid sequence of MCP provided in Fig. 4A (SEQ. ID NO: 5).

MCP works by binding to the C3b and C4b present on the cell surface thereby targeting C3b and C4b for degradation by factor I, a plasma protease, and thereby destroying any subsequent C3 or C4 convertase activity. Thus, MCP is said to have "cofactor activity". Because MCP is localized on the cell surface, it protects only the cells on which it is present and is therefore said to act in an intrinsic manner. The sequence of a cDNA encoding human MCP has been reported by Lublin et al, J. Exp. Med., (1988) 168:181-194. The nucleotide sequence of a cDNA encoding MCP is shown in Fig 4B (SEQ ID NO: 6).

Table 2

Amino Acid	Domain
1-34	Leader peptide
35-95	CCP
96-158	CCP
159-224	CCP
225-285	CCP
286-314	STP
	B-domain: VSTSSTTKPASSAS
	C-domain: GPRPTYKPPVSNP
315-327	Undefined segment
328-351	Transmembrane domain
352-361	Intracytoplasmic anchor
362-377	Cytoplasmic tail one: TYLTDETHREVKFTSL
362-384	Cytoplasmic tail two: KADGGAEYATYQTKSTTPAEQRC

[0015] Effects of Excessive Activation of Complement

[0016] Excessive activation of complement causes damage to normal host tissues in a number of conditions. Some diseases in which complement is known to be activated include systemic lupus erythematosus, acute myocardial infarction, burn, sepsis, stroke and the adult respiratory distress syndrome. Accordingly, it is desirable to have soluble agents that can block complement activation. Such agents would be useful for treating the above-mentioned human diseases and a wide range of other diseases (See Table 3 below). The construction of hybrid complement regulatory proteins has been attempted previously, but with mixed results. For example, a hybrid containing CCPs 1-4 of MCP and CCPs 1-4 of DAF was

constructed by Iwata, et al (*J. Immunol.* 1194, 152:3436). While this hybrid had greater activity in the alternative pathway than either MCP or DAF, it had less activity than DAF alone or DAF plus MCP in the classical pathway. Additionally, in tests of reciprocal chimeric complement inhibitors, one chimeric protein retained the activity of its CD59 and DAF components, while its reciprocal retained only the activity of its DAF component (Fodor, et al, *J.Immunol.*, 1995, 155:4135). Therefore, there is a need for a reliable method for constructing hybrid and chimeric complement regulatory proteins. There is also a need for a hybrid complement regulating protein that possesses the decay accelerating activity of DAF and the co factor activity of CR1.

TABLE 3

Potential Clinical Targets of Protein of the Invention	
Alternative Pathway	Classical Pathway
Reperfusion injury	Autoimmune diseases
Cerebral infarction (stroke)	Systemic lupus erythematosus
Acute myocardial infarction	Rheumatoid arthritis
Hypovolemic shock	Glomerulonephritis
Multiple organ failure	Hemolytic anemia
Crush injury	Myasthenia gravis
Intestinal ischemia	Multiple sclerosis
Inflammatory disorders	Vasculitis
Adult respiratory distress syndrome	Inflammatory bowel diseases
Thermal injury (burn & frostbite)	Bullous diseases
Post-pump syndrome (cardiopulmonary bypass & hemodialysis)	Urticaria
Crohn's disease	Paroxysmal nocturnal
Sickle cell anemia	Hemoglobinuria
Pancreatitis	Cryoglobulinemia
Adverse drug reactions	Inflammatory disorders
Radiographic contrast media allergy	Septic shock & endotoxemia
Drug allergy	Transplant rejection
IL-2 induced vascular leakage syndrome	Hyperacute allograft
Transplant rejection	
Hyperacute xenograft	

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] Fig. 1A is a listing of the amino acid sequence of human Decay Accelerating Factor (DAF), SEQ. ID NO: 1;

[0018] Fig. 1B is a listing of a DNA sequence encoding DAF, SEQ. ID NO: 2;

[0019] Fig. 2 is a listing of the amino acid sequence of human Complement Receptor 1 (CR1), SEQ. ID NO: 3;

[0020] Fig. 3 is a listing of a DNA sequence encoding CR1, SEQ. ID NO: 4;

[0021] Fig. 4A is listing of the amino acid sequence of human Membrane Cofactor Protein (MCP), SEQ. ID NO: 5;

[0022] Fig. 4B is a listing of a DNA sequence encoding MCP, SEQ. ID NO: 6;

[0023] Fig. 5 is a representation of a lipid tail structure;

[0024] Fig. 6A and 6B are listings of PCR primers DSIGEB and DAF3P, SEQ. ID NO's: 7 and 8, respectively;

[0025] Figs. 7A- 7D are listings of PCR primers CR1094X, CR1099N, CR135ON, and CR1B3P, SEQ. ID NO's: 9-12, respectively;

[0026] Fig. 8A is a listing of the amino acid sequence of the protein DAF-CR1B, SEQ. ID NO: 13;

[0027] Fig. 8B is a listing of a DNA sequence encoding DAF-CR1B, SEQ. ID NO: 14;

[0028] Fig. 9A is a listing of the amino acid sequence of protein DAF – CR1BB, SEQ. ID NO: 15;

[0029] Fig. 9B is a listing of a DNA sequence encoding DAF – CR1BB, SEQ. ID NO: 15;

[0030] Fig. 10A and 10B are listings of the PCR primers IgG45 and IgG43, SEQ. ID NO's: 17 and 18, respectively;

[0031] Fig. 11A is a listing of amino acid sequence of protein DAF – IgG4, SEQ. ID NO: 19;

[0032] Fig. 11B is a listing of a DNA sequence encoding DAF – IgG4, SEQ. ID NO: 19;

[0033] Fig. 12A and 12B are listings of the PCR primers MCP5 and MCP3, SEQ. ID NO's: 21 and 22, respectively;

[0034] Fig. 13A is a listing of the amino acid sequence of protein DAF – MCP, SEQ ID NO: 23;

[0035] Fig. 13B is a listing of a DNA sequence encoding DAF – MCP, SEQ. ID NO: 24;

[0036] Fig. 14 is a Western blot of protein samples containing hybrid proteins probed with monoclonal antibodies raised against DAF and CR1;

[0037] Fig. 15 is a Western blot of a protein samples containing DAF – MCP probed with monoclonal antibodies raised against DAF and MCP;

[0038] Fig. 16 is a graph showing the percent inhibition of hemolysis of DAF – CR1BB and sCR1 in a whole serum assay;

[0039] Fig. 17 is a graph showing the percent inhibition of hemolysis of DAF – MCP and DAF in a whole serum assay;

[0040] Figs. 18A and 18B are graphs showing the percent inhibition of hemolysis of the hybrid proteins in a classical pathway C3 convertase assay;

[0041] Fig. 19 is a graph showing the percent inhibition of hemolysis of DAF – CR1B and DAF in a classical pathway C5 convertase assay;

[0042] Fig. 20 is a graph showing the percent inhibition of hemolysis of DAF – CR1BB, sCR1 and DAF-CR1B in a classical pathway C5 convertase assay;

[0043] Fig. 21 is a Western blot of supernatants of cells expressing the hybrid proteins of DAF – MCP or DAF-CR1BB with and without factor I in a cofactor assay.

[0044] Fig 22 is a Western blot of supernatants of cells expressing the hybrid proteins of DAF – Cr1B with and without factor I in a cofactor assay.

SUMMARY OF THE INVENTION

[0045] It is therefore an aspect of the present invention to provide hybrid and chimeric complement regulating proteins. The present invention relates to a family of hybrid and chimeric polypeptides for regulating, more particularly for inhibiting, excessive complement activation.

[0046] A hybrid complement-regulating protein of the present invention comprises a first functional unit of a first complement regulatory protein having complement regulating properties, a first spacer sequence of at least about 200 amino acids encoding a polypeptide that does not exhibit complement regulating properties and at least a second functional unit attached to the spacer sequence. The second functional unit may be a polypeptide providing a functional unit of a second complement regulatory protein, a polypeptide derived from an immunoglobulin, or a polypeptide that enhances binding of the protein to an animal cell. The hybrid protein may also contain a second spacer sequence and a third functional unit of a complement regulatory protein, a polypeptide derived from an immunoglobulin, and a polypeptide that enhances binding of the protein to an animal cell. The optional third functional unit may be the same or different from the first or second functional units. It has been advantageously discovered that the construction of hybrid complement regulating proteins requires more than simply the presence of the protein domains providing decay accelerating activity (as from DAF) and co-factor activity (as from CR1 or MCP). Proper spacing of such domains is also required for activity of both domains in a hybrid protein. In one embodiment, the hybrid protein (referred to hereafter as a "DAF hybrid") comprises CCPs 2 and 3, of DAF as one functional unit. Preferably, such a functional unit also comprises CCP4 and more preferably, comprises CCPs 1-4 of DAF. The DAF hybrid protein can also include one or more functional units that have been derived from CR1, e.g., one or more functional units comprising CCPs 8-10 of CR1 or functional units comprising CCPs 15-17 of CR1, or combinations thereof. The DAF hybrid polypeptide can also include one or more functional units that have been derived from MCP, e.g. CCPs 2, 3, and 4. Preferably, the MCP functional unit also comprises CCP1 of MCP. The DAF hybrid protein can also comprise functional units that have been derived from other complement activation

regulatory proteins. Examples of such proteins include, but are not limited to, the factor H protein and C4BP. In certain embodiments, the hybrid polypeptide comprises functional units that have been derived from three or more complement activation regulatory proteins, in which each functional unit is separated from the preceding functional unit and following functional unit in the hybrid polypeptide by a spacer.

[0047] The present spacer is a polypeptide that is greater than 200 amino acids in length, preferably greater than 250 amino acids in length. Where more than one spacer is used, the amino acid sequences of the spacers that are employed may be the same or different. The spacer may be a synthetic polypeptide fragment. Alternatively, the spacer is derived from a complement activation regulatory protein. In one embodiment, the spacer comprises all or substantially all of CCPs 4-7 of CR1, i.e., amino acid 239 through amino acid 496 of the CR1 sequence shown in Fig. 2 (SEQ. ID NO: 2). These CCPs have no known activity directly associated with them. While not wishing to condition patentability on any particular theory, it is believed that these CCPs function in the native CR1 protein to properly space those CCPs that do have directly-associated activity from each other. In another embodiment, the spacer comprises all or substantially all of CCPs 11-14 of the CR1 protein. In most embodiments, the spacer does not exhibit complement-regulating activity. The hybrid proteins of the present invention are based, at least in part, on Applicants discovery that on an equal molar basis, DAF is at least 4 to 5 times more efficient than LHR A of CR1 in inhibiting the classical pathway.

[0048] The chimeric polypeptides of the present invention comprise at least one functional unit that has been derived from a complement activation regulatory protein (referred to hereinafter as the "first functional unit"), a functional unit that has been derived from a protein that is not a complement activation regulatory protein (referred to hereinafter as the "second functional unit"), and a spacer for separating and appropriately spacing the first functional unit from the second functional unit. The spacer is as described above. The second functional unit can be derived from immunoglobulin (IgG) and may serve to reduce degradation of the chimeric polypeptide following injection into an animal. Alternatively, the second functional unit can be a targeting moiety that enhances binding of the chimeric polypeptide by certain animal tissues. An example of one such targeting moiety is a lipid tail, as shown in attached Figure 5. Such a molecule is expected to target the chimeric polypeptide to the membrane bilayer interior, more particularly to areas of translocated acidic

phospholipid. (See, Smith, RA (2002) Biochem Soc Trans 30 (Pt6):1037-41.) The second function unit can also be a targeting moiety that enhances binding of the chimeric polypeptide to an implant, or to an extracorporeal surface, e.g., a hemodialysis membrane. In certain embodiments, the chimeric protein may comprise multiple functional units that have been derived from one or more complement activation regulatory proteins, each of which are separated from one another by a spacer. Thus, the chimeric polypeptide of the present invention can be a hybrid-chimeric polypeptide, e.g. a polypeptide that comprises a functional unit derived from DAF, a functional unit that has been derived from CR1 and a functional unit that has been derived from IgG 4.

[0049] The present invention also provides isolated polynucleotides that encode the hybrid and chimeric polypeptides of the present invention, constructs formed by inserting an isolated polynucleotide of the present invention into an expression vector, and recombinant host cells into which the constructs of the present invention have been incorporated. In addition to the hybrid and/or chimeric polypeptide encoding sequence, such expression vectors comprise regulatory sequences that control or regulate expression of the polypeptide. Examples of suitable host cells are bacterial cells, yeast cells, insect cells, and mammalian cells. The present invention also relates to a process for preparing the hybrid and/or chimeric proteins of the present invention by culturing the cells of the present invention under conditions that promote expression of the hybrid and/or chimeric protein in the cell. For example, the process may be carried out by expressing the hybrid or chimeric protein in Chinese hamster ovary (CHO) cells or COS cells. The hybrid and chimeric proteins of the present invention may then be collected from a cell culture supernatant or cell lysate of the transformed host cells using an affinity column and then eluting the hybrid and/or chimeric protein from the column.

[0050] The present invention also features methods of reducing inflammation characterized by excessive complement activation in an animal subject. In one aspect, the method comprises administering one or more of the present hybrid polypeptides or chimeric polypeptides to an animal subject, particularly a human subject, afflicted with a condition associated with excessive complement activation. Thus, the present invention also relates to methods of treating patients afflicted with any of the diseases listed in Table 3 below. In another aspect, the present method comprises administering an expression vector comprising

a polynucleotide that encodes a hybrid polypeptide or a chimeric polypeptide of the present invention to the animal subject.

DETAILED DESCRIPTION OF THE INVENTION

[0051] Described herein are hybrid proteins that comprise at least one functional unit of a first complement activation regulatory protein and at least one functional unit of a second complement activation regulatory protein, particularly a protein that inhibits the activity of C3 and/or C5 convertase. In certain embodiments, such hybrid proteins comprise more than one functional unit from a complement activation regulatory protein. In certain embodiments such hybrid proteins comprises functional units from more than 2 complement activation regulatory proteins. The functional units in the present hybrid proteins are separated and spaced apart by a spacer which is described in greater detail below. The functional units can be located in any order within the hybrid proteins of the present invention, provided that proper spacing exists between the functional units.

[0052] I. Hybrid Proteins

[0053] A. DAF Functional Unit

[0054] In certain embodiments, the present hybrid protein preferably comprises at least one functional unit from DAF. Such a functional unit is capable of dissociating C3 and C5 convertases. Thus, the DAF functional unit may comprise CCPs 2 and 3 of DAF, which are sufficient for decay accelerating activity against the classical pathway. Preferably, the DAF functional unit comprise CCPs 2, 3, and 4 of DAF, which are sufficient for decay accelerating activity against both the classical pathway C3 convertase and the alternative pathway C3 convertase. The amino acid sequence of such CCPs may be identical to the native or naturally occurring amino acid sequence of DAF. Alternatively, the amino acid sequence of such CCPs may be altered slightly, particularly at the amino or carboxy terminus. Such alterations occur when a restriction enzyme site is incorporated into the polynucleotide encoding the CCPs. Such alterations also occur when amino acids are deleted from the N terminus or C terminus of the functional unit. (For example, see Example 1 below in which a number of amino acids are deleted from the C terminus of CCP 4 of DAF.) In certain embodiments the hybrid protein may further comprise CCP 1 of DAF. It is also envisioned that some amino acid substitutions in the sequence may be introduced without effecting the

activity of the functional unit as disclosed in U.S. Patent No. 6,521,450, the disclosure of which is hereby incorporated by reference herein. Therefore, the functional unit may have less than complete homology to native protein component. Changes may be made by substitution of like charged amino acids for one another or substitution of hydrophilic amino acids for one another substitution of hydrophobic amino acids for one another and substitution of amino acids of similar mass for one another. In other regions, especially, those unassociated with activity less subtle changes may be made. In one embodiment, the protein contains a functional unit that has at least 95 percent homology to a region of CCPs 1,2,3 or 4 of the human DAF protein. The amino acid sequence of native DAF is shown in Fig. 1A (SEQ. ID NO: 1). CCP1 extends from and includes amino acid 35 through amino acid 95 of the native DAF protein. CCP2 extends from and includes amino acid through amino acid 97 through amino acid 159 of the native DAF protein. CCP3 extends from and includes amino acid 162-221 of the native DAF protein. CCP 4 extends from and includes 224-284 of the native DAF protein. The functional unit also comprises the amino acids that link CCP1 to CCP2, CCP2 to CCP3, and CCP3 to CCP 4 of the DAF protein.

[0055] B. CR1 Functional Units

[0056] The present hybrid protein may also comprise one or more functional units from CR1. Such functional unit is capable of acting as a cofactor for factor I-mediated cleavage of C3b to iC3b and C3f and further cleavage of iC3b to C3c and C3dg. In this way, a hybrid protein may not only have strong activity in dissociating C3 convertase, but also strongly mediate further cleavage of the resulting iC3b protein by factor I. Such a functional unit is also capable of acting as a cofactor for factor I-mediated cleavage of C4b to C4d and C4c. (See K. Yazdanbakhsh et al., Blood, 2003). Thus, the hybrid protein of the present invention may comprise substantially all of CCPs 8-10 and/or CCPs 15-17 of CR1. The amino acid sequence of such CCPs may be identical to the native or naturally occurring amino acid sequence of CCPs 8-10 or CCPs 15-17 of CR1. Alternatively, the amino acid sequence of such CCPs may be altered slightly, particularly at the amino or carboxy terminus. Such alterations occur when a restriction enzyme site is incorporated into the polynucleotide encoding the CCPs 8-10 or CCPs 15-17 of CR1 or when amino acids, preferably a few amino acids, such as 1 to 10 aa's are deleted from the amino terminus or the carboxy terminus or both the amino terminus and the carboxy terminus of the functional unit. Amino acid substitutions, as described above, may also be introduced into the CCPs of CR1. The hybrid

protein of the present invention may comprise two or more functional units from CR1. Such CR1 functional units may be the same or different. Thus, the hybrid protein of the present invention may comprise two functional units derived from LHR-B or one functional unit derived from LHR B and one functional unit derived from LHR-C of the CR1 protein. CCP 8 of CR1 extends from and includes amino acid 497-556 of the native CR1 protein. CCP 9 of CR1 extends from and includes amino acids 557-618 of the native CR1 protein. CCP 10 extends from and includes amino acids 619-688 of the native CR1 protein. CCP 15 of CR1 extends from and includes amino acid 947-1006 of the native CR1 protein. CCP 16 of CR1 extends from and includes amino acid 1007-1068 of the native CR1 protein. CCP 17 of CR1 extends from and includes amino acid 1069-1138 of the native CR1 protein.

[0057] C. MCP Functional Unit

[0058] The present hybrid protein may also comprise one or more functional units from MCP. Such functional unit is capable of acting as a cofactor for factor I-mediated cleavage of C3b to iC3b and C3f. Thus, the hybrid protein of the present invention may comprise substantially all of CCPs 1-4 of MCP. The amino acid sequence of such CCPs may be identical to the native or naturally-occurring amino acid sequence of CCPs 1-4 of MCP. Alternatively, the amino acid sequence of such CCPs may be altered slightly, particularly at the amino or carboxy terminus, or with substitutions as described above. Such alterations occur when a restriction enzyme site is incorporated into the polynucleotide encoding CCPs 1-4 of MCP, or when amino acids are deleted from the amino or carboxy terminus of this functional unit. Preferably, the hybrid protein of the present invention comprises two or more functional units from MCP or units having at least 95 percent homology to two or more functional units from MCP, or alternatively, a functional unit from MCP and a functional unit from CR1. CCP 1 of MCP extends from and includes amino acid 35-95 of the native MCP protein. CCP 2 of MCP extends from and includes amino acid 96-158 of the native MCP protein. CCP3 extends from and includes amino acid 159-224 of the native MCP protein. CCP4 extends from and includes amino acid 225-285 of the native MCP protein.

[0059] D. Spacer

[0060] The hybrid proteins of the present invention comprise one or more spacers. Each spacer in the present hybrid and/or chimeric proteins separate and appropriately space the

functional units of the present hybrid and/or chimeric proteins from one another. Such spacer is a polypeptide that is greater than 200 amino acids in length, preferably greater than 250 amino acids in length. Where more than one spacer is required, the amino acid sequences of the spacers that are employed in the hybrid proteins of the present invention may be the same or different. In one preferred embodiment, the spacer comprises all or substantially all of CCPs 4-7 of CR1, i.e., amino acid 239 through amino acid 496 of the CR1 sequence shown in Fig.2 (SEQ. ID NO: 3). In another preferred embodiment the spacer comprises all or substantially all of CCPs 11-14 of the CR1 protein. As used herein the term substantially all means that the spacer may lack a few, e.g. 1-10 amino acids from the N terminus and/or the C terminus of the spacer. The spacer may also comprise some amino acids that result from incorporating a restriction enzyme site into the spacer. Thus, the spacer may comprise a few amino acids at the N terminus or C terminus that are different from the amino acids that are found at the N terminus or C terminus of the CCP4-7 fragment that is derived from native CR1 or the CCP11-14 fragment that is derived from native CR1. The spacer may also contain substitutions within a sequence as described above, such that the spacer has at least about 95 percent homology to a corresponding native sequence.

[0061] Optionally, the hybrid proteins of the present invention may further include a tag, i.e., a second protein or one or more amino acids, preferably from about 2 to 65 amino acids, that are added to the amino or carboxy terminus of the hybrid protein. Typically, such additions are made to stabilize the protein or to simplify purification of an expressed recombinant form of the hybrid protein. Such tags are known in the art. Representative examples of such tags include sequences which encode a series of histidine residues, the epitope tag FLAG, the Herpes simplex glycoprotein D, beta-galactosidase, maltose binding protein, or glutathione S-transferase.

[0062] The present invention also encompasses hybrid protein proteins in which one or more amino acids are altered by post-translation processes or synthetic methods. Examples of such modifications include, but are not limited to, glycosylation, iodination, myristoylation, and pegylation.

[0063] II. Chimeric Proteins.

[0064] The chimeric proteins of the present invention comprise one or more functional units of complement activation regulatory protein as described above and one or more functional units derived from a protein that is not a complement activation regulatory protein. Examples of functional units that are not derived from complement activation regulatory proteins include functional units that are derived from an immunoglobulin, particularly IgG4, and that serve to reduce degradation of the chimeric polypeptide following injection into an animal. Thus, the chimeric protein may include the hinge, CH2, and CH3 domains of IgG4. Alternatively, the second functional unit can be a targeting moiety that enhances binding of the chimeric polypeptide by certain animal tissues. An example of such targeting moiety is a lipid tail, as shown in attached Figure 5. In certain embodiments, the chimeric protein may comprise multiple functional units that have been derived from one or more complement activation regulatory proteins, each of which are separated from one another by a spacer. Thus, the chimeric polypeptides of the present invention can be a hybrid, chimeric polypeptide.

[0065] Preparation of the Hybrid and Chimeric Proteins

[0066] The present hybrid proteins and chimeric proteins of the present invention are prepared using polynucleotides that encode such proteins and expression systems.

[0067] The functional units and spacers employed in the present hybrid and/or chimeric proteins can be made by obtaining total (t) or messenger (m) RNA from an appropriate tissue, cell line or white blood cells. Suitable RNA (total or messenger) is also available commercially. Blood can be drawn from a human or other animal subject and peripheral blood mononuclear cells (PBMCs) can be purified by Ficoll-Paque density centrifugation. Total RNA from PBMCs should contain CR1 and IgG4. Cell lines can be grown in controlled climate incubators with appropriate cell culture media. DAF and MCP are fairly ubiquitous proteins. Thus, these proteins can be found in most cell lines, e.g., the HeLa cell line.

[0068] Following isolation of suitable RNA, the RNA is reverse transcribed to cDNA using commercially available reagents and standard protocols, e.g., the Superscript protocol of Invitrogen. Once the appropriate cDNA is made, polymerase chain reaction (PCR) can be

used in conjunction with DNA polymerases and oligonucleotide primer pairs (20 to 30 nucleotides in length) to amplify DAF, MCP, CR1 and/or IgG4 cDNA. One primer will be at the 5' end of the cDNA (for example at the start codon ATG or, in the case of the constant heavy chain, further downstream at the start of the constant heavy region 1 [CH1] and one primer will be at the 3' end of the cDNA, e.g. at the stop codon TAG, TAA, or TGA). The PCR products are then subcloned into vectors such as pT7Blue (pT7B) (Novagen, Madison, WI) and sequenced to confirm that the correct cDNA was amplified.

[0069] Expression Systems For Producing The Hybrid Proteins

[0070] The present hybrid proteins can be produced in procaryotic and eucaryotic cells each using different expression vectors that are appropriate for each host cell. Eucaryotic expression system such as the baculoviral or mammalian cells are described below.

[0071] The following are examples of expression vectors which may be used for gene expression in an eucaryotic expression system. The plasmid, pMSG, uses the promoter from mouse mammary tumor virus long terminal repeat (MMTV). Suitable host cells for pMSG expression are chinese hamster ovary (CHO) cells, HeLa cells and mouse Lkt negative cells (Lee, F., et al., 1981, Nature 294:228-232). The vector, pSVL, uses the SV40 late promoter. For high transient expression, suitable host cells for pSVL expression are COS cells (Sprague, J. et al., 1983, J. Virol. 45:773-781). The vector, pRSV, uses Rous Sarcoma Virus promoter. Suitable host cells for pRSV expression are mouse fibroblast cells, lymphoblastoid cells and COS cells (Gorman, Padmanabhan and Howard, 1983, Science 221:551-553).

[0072] Baculovirus expression vectors can also be used. These vectors are stably expressed in insect cells such as sf9 (Luckow, V. A. and Summers, M. D., 1988, Bio/Technology 6:47-55; Miller, L. K., 1988, Ann. Re. Microbiology 42:177-199).

[0073] Hybrid proteins of the invention can also be produced in a procaryotic expression system. The following are examples of expression vectors which can be expressed in procaryotic expression systems. The pOX expression series using the oxygen-dependent promoter can be expressed in E. coli. (Khosla, G., et al., 1990, Bio/Technology 8:554-558). pRL vector which uses the strong pL promoter of lambda phage (Reed, R. R., 1981, Cell 25:713-719; Mott, J. D., et al., 1985, Proc. Natl. Acad. Sci. U.S.A. 82:88-92) and the pKK223-3 vector which uses a hybrid promoter derived from the fusion between the

promoters of the tryptophan and lactose operons of *E. coli*. (Borsius, J. and Holy, A., 1984, *Proc. Natl. Acad. Sci. U.S.A.* 81:6929-6933) can be used for expression in *E. coli*.

[0074] Suitable vectors for yeast expression are also well known in the art, e.g., Sleep, D., Belfield, D. P. and Goodey, A. R., 1990, *Bio/Technology* 8:42-46; Sakai, A. et al., 1991, *Bio/Technology* 9:1382-1385; Kotula, L. and Curtis, P. J., 1991, *Bio/Technology* 9:1386-1389, all of which are herein incorporated by reference.

[0075] Production, Quantitation, Purification And Analysis Of The Hybrid Proteins.

[0076] Once a recombinant cell line that expresses the hybrid protein has been isolated, the secreted proteins are identified and verified with regard to their predicted structure. Various methods can be used to identify and characterize the expressed hybrid proteins. The presence of secreted hybrid proteins can be verified by immunoprecipitation with monoclonal antibodies to one or the other fragment, e.g., antibodies that bind to CCP's, 2, 3, or 4 of DAF or to LHR B or C of CR1.

[0077] Another method that could be used with present hybrid and/or chimeric proteins is a double immunoprecipitation, using two monoclonal antibodies of different specificities in succession. Pre-clearance of culture supernatant with one antibody would result in a negative immunoprecipitation with the second antibody. This method would verify that a single protein expresses both CR1 and DAF epitopes.

[0078] Alternatively, the hybrid DAF-CR1 protein, can be identified by Western blot. For example, after SDS-PAGE and transfer to nitrocellulose, blots can be developed with either anti-CR1 antibodies or anti-DAF monoclonal antibodies. The expressed bispecific recombinant protein would be reactive with both antibodies, again demonstrating the presence of specific DAF and CR1 epitopes in the hybrid protein.

[0079] Identification of the present hybrid and/or chimeric proteins can also be accomplished by ELISA. For example, a rabbit polyclonal antibody specific for either LHR B or C of CR1 or CCP's 2, 3, or 4 of DAF can be used to coat plastic microtiter ELISA plates, followed by the addition of culture supernatant from the recombinant cell line expressing the DAF-CR1 hybrid and incubation with the capture polyclonal antibody. A monoclonal anti-DAF or anti-CR1 second antibody, the specificity of which is different from the capture antibody, can be

subsequently used. A positive reaction would indicate the presence of both epitopes on the hybrid or chimeric protein.

[0080] An ELISA can also be used to quantitate the levels of the DAF-CR1 hybrid protein in culture supernatants or any other unpurified solutions containing the chimeric protein by comparison to standard curve of known quantities of purified DAF-CR1 hybrid protein. Quantitation of DAF-CR1 hybrid protein would be useful for determination of production rates in recombinant cell lines, determination of protein concentration in partially purified preparations, and for determination of protein concentration in plasma for in vivo experiments.

[0081] The hybrid and/or chimeric proteins of the present invention can be purified from recombinant cell culture supernatant by a variety of standard chromatographic procedures, including but not limited to immunoaffinity chromatography, ion exchange chromatography, gel filtration chromatography, reverse-phase high pressure liquid chromatography (HPLC), lectin affinity chromatography, or chromatofocusing. For example, small quantities of culture supernatant containing serum supplement can be purified using immunoaffinity chromatography with, e.g., anti-CR1 or anti-DAF monoclonal antibodies. DAF-CR1 hybrid protein bound to the immobilized antibody can be eluted in purified form by use of a chaotropic solution.

[0082] Once the hybrid and/or chimeric protein is purified, its amino acid sequence can be deduced by direct protein sequence analysis using an automated system. The presence of N- and O-linked carbohydrates can be determined by use of specific endoglycosidase enzymes (Chavira, R. et al., 1984, Anal. Biochem. 136:446). Further characterizations of its biochemical structure can also be performed, including but not limited to pI determination by isoelectric focusing, hydrophilicity analysis, X-ray crystallographic analysis, and computer modeling.

[0083] Functional Characterization Of The Present Hybrid And/Or Chimeric Proteins

[0084] The hybrid proteins of the present invention have the ability to function both as a cofactor for Factor I and as a decay accelerating factor. In vitro assays can be performed to measure these biological activities (Medof, M. et al., 1984, J. Exp. Med. 160:1558; Masaki, T. et al., 1992, J. Biochem 111:573). As described in the examples, assays for cofactor

activity and for decay accelerating activity are used to demonstrate both these complement regulatory functions for the present hybrid protein. The consequence of either cofactor or decay accelerating activity, or in the case of a DAF-CR1 or DAF-MCP hybrid protein, both activities in combination, is the inactivation of C3/C5 convertases. Another suitable in vitro assay demonstrates that the present hybrid protein is capable of inhibiting C5 convertase activity as measured by the production of C5a (Moran, P. et al., 1992, J. Immunol. 149:1736, herein incorporated by reference). Additional assays, as described in the examples below, demonstrate that the present hybrid proteins inhibit the complement-induced lysis of cells via the classical and alternative pathways.

[0085] Demonstration Of In Vivo Therapeutic Activity Of The Present Hybrid And/Or Chimeric Proteins

[0086] The Arthus reaction is an inflammatory response caused by the interaction of antigen in tissue with circulating antibody. It has been used as a classic example of a localized in vivo inflammatory response, and is characterized by the formation of immune complexes, complement activation, inflammatory cell recruitment, edema and tissue damage (Bailey, P. and Sturm, A., 1983, Biochem. Pharm 32:475). Experimentally, a reversed passive Arthus reaction can be established in an animal model by i.v. injection with antigen and subsequent challenge with antibody. Using guinea pigs as an animal model, the in vivo therapeutic efficacy of the hybrid and/or chimeric proteins of the invention can be evaluated.

[0087] Additional animal models with relevance to various clinical human diseases can also be used to test the in vivo efficacy of complement activation blockers. These include, but are not limited to: myocardial ischemia/reperfusion injury (acute myocardial infarction; Weisman, H. F. et al., 1990, Science 249:146); cerebral ischemic injury (stroke; Chang, L. et al., 1992, J. Cerebr. Blood Flow Metab. 12:1030); lung injury (ARDS; Hosea, S. et al., 1980, J. Clin. Invest. 66:375); xenograft rejection (transplants; Leventhal, J. et al., 1993, Transplantation 55:857); burn injury (Caldwell, F, et al., 1993, J. Burn Care Rehab. 14:420); acute pancreatitis (Steer, M. 1992, Yale J. Biol. Med. 65:421), nephritis (Pichler, R. et al., 1994, Am. J. Pathol. 144:915), cardiopulmonary bypass (Nilsson, L. et al., 1990, Artif. Organs 14:46), and multiple sclerosis (Linington, C. et al., 1989, Brain 112:895).

[0088] Administration of the Present Hybrid and Chimeric Proteins to Animal Subjects

[0089] The present hybrid and chimeric proteins can be combined with an appropriate pharmaceutical formulation and administered to an animal subject, particularly a human subject, by a variety of routes, including, but not limited to, intravenous bolus injection, intravenous infusion, intraperitoneal, intradermal, intramuscular, subcutaneous, and intranasal routes. The administration of the present hybrid proteins in vivo will enable the protein to bind endogenous C3/C5 convertases and inhibit the generation of additional C3b and C5b, of C3a and C5a anaphylatoxins, and of C5b-9 lytic complexes. The complement regulatory activities of the present hybrid proteins can therefore function to inhibit in vivo complement activation and the inflammatory sequelae that accompany it, such as neutrophil recruitment and activation, autolysis of host cells, and edema. The present hybrid and/or chimeric proteins can be used for the therapy of diseases or conditions that are mediated by inordinate and/or excessive activation of the complement system. These include, but are not limited to: tissue damage due to ischemia-reperfusion following myocardial infarction, aneurysm, stroke, hemorrhagic shock, or crush injury; burns; endotoxemia and septic shock; adult respiratory distress syndrome (ARDS); hyperacute rejection of grafts; cardiopulmonary bypass and pancreatitis. Autoimmune disorders including, but not limited to, systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis, can also be treated with the hybrid and/or chimeric proteins of the invention (also see Table 3).

[0090] Various delivery systems are known and can be used to deliver the hybrid and/or chimeric proteins of the invention, such as encapsulation in liposomes, or controlled release devices. The hybrid and/or chimeric proteins of the invention can also be administered extracorporeally, e.g., pre-conditioning donor organs prior to transplantation. The hybrid and/or chimeric proteins of the invention can be formulated in a pharmaceutical excipient in the range of approximately 10 µg/kg and 10 mg/kg body weight for in vivo or ex vivo treatment.

[0091] Administration of Polynucleotides That Encode the Present Hybrid and/or Chimeric Proteins to an Animal Subject

[0092] The present invention also relates to therapeutic methods in which polynucleotides that encode and express the present hybrid and/or chimeric polypeptides are introduced into a

subject in need of the same, i.e. a subject, particularly a human subject, with a disorder associated with increased complement activation. Polynucleotides encoding and expressing one or more hybrid and/or chimeric polypeptide can be introduced into cells of the subject using any of a variety of methods known in the art to achieve transfer of DNA molecules into cells. For example, DNA encoding and expressing the hybrid and/or chimeric polypeptide can be incorporated into liposomes and targeted to and internalized by the cells of the subject. Polynucleotides encoding the hybrid and/or chimeric polypeptide can also be incorporated into plasmids that are introduced into cells of the subject by transfection. The hybrid and/or chimeric polypeptide encoding polynucleotides can also be introduced into cells using viruses. Such viral "vectors" can have DNA or RNA genomes. Numerous such viral vectors are well known to those skilled in the art. Viral vectors that have polynucleotide sequences encoding a DAF-CR1 hybrid polypeptide, for example, cloned into their genomes are referred to as "recombinant" viruses. Transfer of DNA molecules using viruses is particularly useful for transferring polynucleotide sequences into particular cells or tissues of an animal. Such techniques are commonly known in the art as gene therapy.

[0093] Expression vectors normally contain sequences that facilitate gene expression. An expression vehicle can comprise a transcriptional unit comprising an assembly of a protein encoding sequence and elements that regulate transcription and translation. Transcriptional regulatory elements generally include those elements that initiate transcription. Types of such elements include promoters and enhancers. Promoters may be constitutive, inducible or tissue specific. Transcriptional regulatory elements also include those that terminate transcription or provide the signal for processing of the 3' end of an RNA (signals for polyadenylation). Translational regulatory sequences are normally part of the protein encoding sequences and include translational start codons and translational termination codons. There may be additional sequences that are part of the protein encoding region, such as those sequences that direct a protein to the cellular membrane, a signal sequence for example.

[0094] The hybrid and/or chimeric polypeptide encoding polynucleotides that are introduced into cells are preferably expressed at a high level (i.e., the introduced polynucleotide sequence produces a high quantity of the hybrid and/or chimeric polypeptide within the cells) after introduction into the cells. Techniques for causing a high-level of expression of polynucleotide sequences introduced into cells are well known in the art. Such techniques

frequently involve, but are not limited to, increasing the transcription of the polynucleotide sequence, once it has been introduced into cells. Such techniques frequently involve the use of transcriptional promoters that cause transcription of the introduced polynucleotide sequences to be initiated at a high rate. A variety of such promoters exist and are well known in the art. Frequently, such promoters are derived from viruses. Such promoters can result in efficient transcription of polynucleotide sequences in a variety of cell types. Such promoters can be constitutive (e.g., CMV enhancer/promoter from human cytomegalovirus) or inducible (e.g., MMTV enhancer/promoter from mouse mammary tumor virus). A variety of constitutive and inducible promoters and enhancers are known in the art. Other promoters that result in transcription of polynucleotide sequences in specific cell types, so-called "tissue-specific promoters," can also be used. A variety of promoters that are expressed in specific tissues exist and are known in the art. For example, promoters whose expression is specific to neural, liver, epithelial and other cells exist and are well known in the art. Methods for making such DNA molecules (i.e., recombinant DNA methods) are well known to those skilled in the art.

[0095] In the art, vectors refer to nucleic acid molecules capable of mediating introduction of another nucleic acid or polynucleotide sequence to which it has been linked into a cell. One type of preferred vector is an episome, i.e., a nucleic acid capable of extrachromosomal replication. Other types of vectors become part of the genome of the cell into which they are introduced. Vectors capable of directing the expression of inserted DNA sequences are referred to as "expression vectors" and may include plasmids, viruses, or other types of molecules known in the art.

[0096] Typically, vectors contain one or more restriction endonuclease recognition sites which permit insertion of the hybrid polypeptide encoding sequence. The vector may further comprise a marker gene, such as for example, a dominant antibiotic resistance gene, which encode compounds that serve to identify and separate transformed cells from non-transformed cells.

[0097] One type of vector that can be used in the present invention is selected from viral vectors. Viral vectors are recombinant viruses which are generally based on various viral families comprising poxviruses, herpesviruses, adenoviruses, parvoviruses and retroviruses. Such recombinant viruses generally comprise an exogenous polynucleotide sequence (herein,

a polynucleotide encoding the hybrid and/or chimeric polypeptide) under control of a promoter which is able to cause expression of the exogenous polynucleotide sequence in vector-infected host cells.

[0098] One type of viral vector is a defective adenovirus which has the exogenous polynucleotide sequence inserted into its genome. The term "defective adenovirus" refers to an adenovirus incapable of autonomously replicating in the target cell. Generally, the genome of the defective adenovirus lacks the sequences necessary for the replication of the virus in the infected cell. Such sequences are partially or, preferably, completely removed from the genome. To be able to infect target cells, the defective virus contains sufficient sequences from the original genome to permit encapsulation of the viral particles during *in vitro* preparation of the construct. Other sequences that the virus contains are any such sequences that are said to be genetically required "in *cis*."

[0099] Preferably, the adenovirus is of a serotype which is not pathogenic for man. Such serotypes include type 2 and 5 adenoviruses (Ad 2 or Ad 5). In the case of the Ad 5 adenoviruses, the sequences necessary for the replication are the E1A and E1B regions. Methods for preparing adenovirus vectors are described in U.S. Patent No. 5,932,210, U.S. Patent No. 5,985,846, and U.S. Patent No. 6,033,908.

[00100] More preferably, the virus vector is an immunologically inert adenovirus. As used herein the term "immunologically inert" means the viral vector does not encode viral proteins that activate cellular and humoral host immune responses. Methods for preparing immunologically inert adenoviruses are described in Parks et al., *Proc Natl Acad Sci USA* 1996; 93(24) 13565-70; Leiber, A. et al., *J. Virol.* 1996; 70(12) 8944-60; Hardy s., et al, *J. Virol.* 1997, 71(3): 1842-9; and Morsy et al, *Proc. Natl. Acad. Sci. USA* 1998. 95: 7866-71, all of which are specifically incorporated herein by reference. Such methods involve Cre-*loxP* recombination. In vitro, Cre-*loxP* recombination is particularly adaptable to preparation of recombinant adenovirus and offers a method for removing unwanted viral nucleotide sequences. Replication deficient recombinant adenovirus lacks the E1 coding sequences necessary for viral replication. This function is provided by 293 cells, a human embryonic kidney cell line transformed by adenovirus. First generation adenoviruses are generated by co-transfecting 293 cells with a helper virus and a shuttle plasmid containing the foreign gene of interest. This results in the packaging of virus that replicates both the foreign gene and

numerous viral proteins. More recently, 293 cells expressing Cre recombinase, and helper virus containing essential viral sequences and with a packaging signal flanked by *loxP* sites, have been developed (See Parks et al.) In this system, the helper virus supplies all of the necessary signals for replication and packaging *in trans*, but is not packaged due to excision of essential sequences flanked by *loxP*. When 293-Cre cells are co-transfected with this helper virus, and a shuttle plasmid (pRP1001) containing the packaging signal, nonsense "filler DNA", and the foreign gene, only an adenovirus containing filler DNA and the foreign gene is packaged (LoxAv). This results in a viral recombinant that retains the ability to infect target cells and synthesize the foreign gene, but does not produce viral proteins.

[00101] Another type of viral vector is a defective retrovirus which has the exogenous polynucleotide sequence inserted into its genome. Such recombinant retroviruses are well known in the art. Recombinant retroviruses for use in the present invention are preferably free of contaminating helper virus. Helper viruses are viruses that are not replication defective and sometimes arise during the packaging of the recombinant retrovirus.

[00102] Non-defective or replication competent viral vectors can also be used. Such vectors retain sequences necessary for replication of the virus. Other types of vectors are plasmid vectors.

[00103] The methods also involve introduction of polynucleotides encoding the present hybrid and/or chimeric polypeptides into an animal subject in the context of cells (e.g., *ex vivo* gene therapy).

[00104] EXAMPLES

[00105] The following examples contained herein are intended to illustrate but not limit the invention.

[00106] EXAMPLE 1: Hybrid Protein DAF - CR1B

[00107] A hybrid protein, DAF - CR1B, comprising a decay accelerating functional unit derived from DAF, a cofactor 1 functional unit derived from CR1, and a spacer comprised of CCPs 4-7 of CR1 was made by recombinant techniques. The DAF portion of

the hybrid protein DAF - CR1B was constructed using *DAF13.2.l/pBTKS* and two primers *DSIGEB* and *DAF3P* in a PCR reaction (Vent polymerase [New England Biolabs] with the following times: 94°C 3min [initial melting]; 94°C 1min, 55°C 1min15sec, 72°C, 1min15sec for 25 cycles; and 72°C 7min [final extension]). *DSIGEB* is a 42 nucleotide ("nt") primer that has the sequence 5'-ATA TAC GAA TTC AGA TCT ATG ACC GTC GCG CGG CCG AGC GTG-3' (Fig. 6A SEQ. ID NO:7). *DAF3P* is a 35nt primer that has the sequence 5'-ACA GTG CTC GAG CAT TCA GGT GGT GGG CCA CTC CA-3' (Fig. 6B, SEQ. ID NO:8). The resultant PCR product was named *DAF1*. It contained DAF's signal sequence followed by CCPs 1, 2, 3 and 4 ending with cysteine 249 (Cys-249) in CCP4. Upstream of the signal sequence, two restriction enzyme sites were built in, BglII (A▼GATCT) and 5' of BglII, EcoRI (G▼AATTC). Three prime (3') of CCP4 and encompassing part of the Cys-249 codon (TGC), the restriction enzyme site XhoI (C▼TCGAG) was inserted. *DAF1* was subcloned into *pT7B* and fully sequenced.

[00108] The CR1 portion of the hybrid protein DAF - CR1B was constructed using *CR1/AprM8*. *CR1/AprM8* was cut with the restriction enzyme NsiI (ATGCA▼T) releasing several pieces, two of which were recovered (1094nts and 1350nts) and subcloned into *pGEM7Zf(+)*. The "1094" fragment (encompassing nts.557 to 1670 of CR1) was amplified by PCR using the primers *CR1094X*(5') and *CR1094N*(3'). *CR1094X* is a 41nt primer having the sequence 5'-ATA TAC CTC GAG TCC TAA CAA ATG CAC GCC TCC AAA TGT GG-3' (Fig. 7A, SEQ ID NO:9). It has an XhoI site. *CR1094N* is a 34nt primer having the sequence 5'-ACA GTG ATG CAT TGG TTT GGG TTT TCA ACT TGG C-3' (Fig 7B, SEQ ID NO:10). It has an NsiI site. This set of primers produces a sequence from the linker between CCP3 and CCP4 of CR1 into CCP8 of CR1. PCR conditions were the same as those for *DAF1*. The "1350" fragment, encompassing nts 1671 to 3020 of CR1, was amplified by PCR using primers *CR1350N*(5') and *CR1B3P*(3'). *CR1350N* is a 41nt primer having the sequence 5'-ATA TAC ATG CAT CTG ACT TTC CCA TTG GGA CAT CTT TAA AG-3' (Fig. 7C, SEQ ID NO: 11). It has an NsiI site. *CR1B3P* is a 57nt primer having the sequence 5'-ACA GTG AGA TCT TTA GTG ATG GTG ATG GTG ATG AAT TCC ACA GCG AGG GGC AGG GCT-3' (Fig. 7D, SEQ ID NO: 12). It has a BglII site. PCR conditions were the same as those for *DAF1* except the 25 cycle extension time at 72° was 2min, not 1min15sec. This set of primers produces a sequence from CCP8 of CR1 to the end of CCP14 (in LHRB, specifically, ...SSPAPRCGI) with a C-terminal 6XHis tag and stop codon. These PCR fragments were subcloned into *pT7B*.

[00109] It is noteworthy that the natural linker between CCP3 and CCP4 of CR1 is the amino acid sequence "IIPNK" (see Fig. 2, SEQ. ID NO: 3). Due to the insertion of the XhoI restriction site, the hybrid protein's linker between DAF CCP4 and CR1 CCP4 is "SSPNK" (see Fig. 8A (SEQ. ID NO: 13).

[00110] DNA sequence the data obtained confirmed the presence of the correct nucleotide sequences.

[00111] The vector pSG5 (Stratagene) was cut with the restriction enzymes EcoRI and BglII to accommodate the insertion of *DAF1* (EcoRI to XhoI), *CR828XN3* (XhoI to NsiI) and *CR1300NBF* (NsiI to BglII). The vector and the three fragments were ligated using Promega T4 DNA ligase, and transformed into DH5 α maximum efficiency competent cells. Agarose gel electrophoresis confirmed the presence of the vector and insert. The cDNA from one colony was used for transfection into COS cells using Lipofectamine (Invitrogen) reagent. The supernatant was harvested two days later. Western blots using 2H6, an anti-DAF CCP4 antibody, and an anti-His tag antibody indicated the presence of the hybrid protein. The amino acid sequences of the DAF - CR1B is provided in Fig. 8A (SEQ. ID NO: 13). A DNA sequence encoding DAF - CR1B is provided in Fig. 8B (SEQ. ID NO: 14).

[00112] EXAMPLE 2: Hybrid Protein DAF - CR1BB

[00113] Another hybrid protein, DAF - CR1BB, was prepared by recombinant techniques. DAF - CR1BB, comprises DAF's four CCPs, a spacer comprised of CCPs 4-7 of CR1, separating the functional unit of DAF from a first cofactor 1 functional unit of CR1, LHR B, and a second spacer, CCPs 11-14 of CR1, separating the first cofactor 1 functional unit of CR1 from the second cofactor 1 functional unit of CR1, also LHR B. More specifically, DAF - CR1BB was prepared by adding an additional LHRB of CR1 to DAF - CR1B. To add the additional cofactor LHR, DAF - CR1B was cut with BamHI and a BamHI fragment (nucleotide #1861 to 3210) from CR1 in AprM8 was introduced. The BamHI fragment could enter the plasmid in either the correct or reverse orientation. Screening with SmaI found several clones with the correct nucleotide orientation. The amino acid sequence of DAF - CR1BB is provided in Fig 9A (SEQ ID NO: 15). The DNA sequence encoding DAF - CR1BB is provided in Fig 9B (SEQ ID NO: 16)

[00114] EXAMPLE 3: Chimeric Protein DAF -Ig G4

[00115] To increase half life of the hybrid protein, while minimizing complement activation, as a starting point, part of the constant heavy region of IgG4 was amplified by PCR and connected 3' to nucleotides encoding a decay accelerating functional unit derived from DAF, and a spacer derived from CR1. The resulting protein DAF - IgG4, is composed of DAF CCPs1,2,3,4 + CR1 CCP4,5,6,7 (part of LHRA) + IgG4, last amino acid (valine) of CH1- Hinge-CH2-CH3. The domains of IgG4 were amplified by PCR from pHc-huG4, a gift of Gary McLean, 2222 Health Sciences Mall, Vancouver, B.C., Canada. The primers used in this PCR reaction were:

IgG45: 5'-ATA TAC GAA TTC TGG TTG AGT CCA AAT ATG GTC CC-3' (Fig 10A (SEQ ID NO: 17)) and

IgG43: 5'-ACA GTG AGA TCT TTA TCA TTT ACC CGG AGA CAG GGA G-3' (Fig 10B (SEQ ID NO: 18)).

[00116] Per 100µl PCR reaction, 2U Vent polymerase (New England Biolabs), 71.5ng pHc-Cg4 (7157 bp), 50 pmol of each primer, and 10mM of each dNTP were used. PCR settings were 1 initial denaturing cycle of 94°C 3 min; 25 cycles of 94°C 1 min, 55°C 1 min 15 sec, and 72°C 1 min; and a final extension cycle of 72°C 7 min. A 700 bp fragment was recovered with the QIAquick gel purification kit. The fragment "IgG4 PCR" was ligated into the pT7Blue (pT7B) blunt vector (Novagen). Nova Blue (Novagen) and XL1Blue (Stratagene) competent cells were transformed with the ligation mixture and plated on Ampicillin 50, Tetracycline 15, IPTG/Xgal LB agar plates. DNA sequence data confirmed the presence of the correct nucleotide sequence. A DNA sample was subsequently cut with EcoRI ("E") and BglII ("B") in Promega Buffer H ("H") and the 700bp band was purified with QIAquick. IgG41 E/B and pSG5 E/B/H were ligated using the Quick Ligation method (New England Biolabs) and transformed into DH5αmax (Invitrogen) competent cells. The presence of the correct inserts was confirmed by digestion with EcoRI and BglII in Buffer H (Promega).

[00117] The resulting plasmid was cut with EcoRI (~4800bp linearized), purified, shrimp alkaline phosphatase ("SAP")-treated, purified again, and quick ligated to an/EcoRI-cut fragment (gel purified, 1650bp) which contains the DAF and CR1 portions of the

sequence. DH5 α max competent cells were transformed with the ligation mix. Plasmid DNA from resulting colonies were screened by cutting the DNA with BglII and examining the resulting band by agarose gel electrophoresis. Plasmid DNA was purified and cDNA was checked (uncut and BglII-cut). The amino acid sequence of DAF - IgG4 is provided in Fig 11A (SEQ. ID NO: 19). The DNA sequence encoding DAF - IgG4 is provided in FIG 11B (SEQ. ID NO: 20). The cDNA was transfected into COS cells.

[00118] Note that the IgG45 primer codes for a slightly different link between CR1 CCP7 and the Hinge of IgG4 (in DAF - IgG4) than the link between CR1 CCP7 and MCP CCP1 (in "DAF - MCP" see Example 4). IgG45 results in "GILV" ("V" is the last amino acid of the CH1 domain of IgG4) instead of "GILGH" which is found in DAF - MCP and is also the normal link between CR1 CCP7 and CCP8 (and is therefore what is found in DAF - CR1B and DAF - CR1BB hybrids).

[00119] EXAMPLE 4 Hybrid Protein DAF - MCP

[00120] A hybrid protein, referred to hereafter as DAF -MCP, comprising a decay accelerating functional unit of DAF, a cofactor 1 functional unit derived from MCP, and a spacer derived from CR1 was prepared (DAF CCPs 1,2,3,4- CR1 CCPs 4,5,6,7- MCP CCPs 1,2,3,4+ 2 amino acids (VS) of MCP STP region + 6xHis). MCP cDNA (with 3'-end sequence encoding GPI-anchor addition) in PEE14 was used. More MCP cDNA in DH5 α (Wizard SV DAF - IgG4-prep) ("MCP-GPI (A)") was subsequently prepared. Primers for the MCP portion of DAF -MCP are:

MCP5: 5'-ATA TAC GAA TTC TGG GTC ACT GTG AGG AGC CAC CAA CAT TTG AAG C-3' (Fig. 12A, SEQ ID NO: 21); and

MCP3: 5'-ACA GTG AGA TCT TTA GTG ATG GTG ATG GTG ATG CGA CAC TTT AAG ACA CTT TGG AAC-3' (Fig. 12B, SEQ. ID NO: 22).

[00121] The PCR reaction used Vent polymerase from New England Biolabs. The MCP PCR fragment was cut with EcoRI ("E") and BglII ("B") in Promega Buffer H ("H"). The Quick Ligase method (New England Biolabs) was used to ligate E/B/H-cut MCP PCR and E/B/H-cut pSG5. DH5 α maximum efficiency competent cells were transformed with the mixture. Colonies were picked, the DNA was extracted from the bacteria and cut with

E/B/H. All had an insert. Two DNA samples were sequenced. An error at Nucleotide 680 was found which changes the amino acid at that position. Primers were made to perform site-directed mutagenesis to correct the error (not shown). Subsequent DNA sequence data confirmed the correct sequence.

[00122] The resulting DNA was cut with EcoRI in Buffer H and purified with Qiagen PCR purification kit. The purification product was treated with shrimp alkaline phosphatase ("SAP"), and purified again. Quick ligation method was used to ligate EcoRI- and SAP-treated MCP1A/pSG5 and EcoRI-treated and gel purified 1650 base pair piece from DAF - CR1BA which adds the DAF and CR1 portions of the cDNA. After transformation, plasmid DNA was cut with BglII or with EcoRI to confirm the presence and correct orientation of the insert. The amino acid sequence of DAF - MCP is provided in Fig 13A (SEQ. ID NO: 23). A DNA sequence of DAF -MCP is provided in Fig. 13 B (SEQ. ID NO: 24).

[00123] DNA was used for COS cell transfection. DAF - MCP 5A protein was visualized by western blot with monoclonal antibodies IA10 (against DAF CCP1) and GB24 (against MCP CCPs 3 and 4) (not shown). Size was between 90kDa and 100kDa.

[00124] Testing of the Hybrid Proteins

[00125] Hemolytic Assays

[00126] Hemolytic assays are performed to assess the activity of the components of the classical and alternative pathways of complement or the activity of the regulators of complement activation (RCA) proteins. Classical pathway activity is assessed using antibody-sensitized sheep erythrocytes (EshA) and can be undertaken in a variety of ways. Whole serum can be used, or purified components of the complement cascade can be used in a classical pathway C3 or C5 convertase hemolytic assay.

[00127] Experiments were undertaken using normal human, pig and rat serum to assess animal models for study. Human DAF has been found to be active against convertases formed in pig serum (J.M. Perez de la Lastra *et al.* 2000, J. Immunol. 165:2563). It is active at high concentrations against convertases formed in rat serum (C.L. Harris *et al.*, 2000, Immunology 100:462). In contrast, human CR1 is highly active against convertases formed in rat serum. Classical pathway C3 convertase and C5 convertase assays utilizing purified

human complement components were also done to compare the hybrids' performance to soluble DAF and soluble CR1.

[00128] Whole Serum Experiments

[00129] Serum (human, pig or rat) was titrated to a Z score of approximately 1, defined as an average of 1 lesion per sheep cell (A.P. Gee, 1983, "Molecular Titration of Components of the Classical Complement Pathway" in Methods in Enzymology 93:339). To each tube was added 1×10^7 EshA, an RCA protein and the serum in DGVB⁺⁺ (dextrose gelatin veronal buffer with calcium and magnesium) to a total volume of 200ul. This mixture was shaken for 30min in a 37°C water bath. GVBE (gelatin veronal buffer with EDTA) was subsequently added to stop the hemolytic reaction. The extent of lysis was determined by reading the OD₄₁₂ of the hemoglobin red supernatant.

[00130] Classical Pathway C3 Convertase Hemolytic Assay

[00131] EshA (1×10^7 cells/tube) were sequentially shaken and spun down with 30SFU (site-forming units) of human C1 (ART) for 15min, 15SFU of human C4 (Quidel) for 20min, and sufficient human C2 (ART) for a Z score of 1 for 5min, all at 30°C. Following formation of the classical pathway C3 convertase C4b2a, regulators were added for 15min at 30°C to assess their relative decay-accelerating activity. Guinea pig serum in GVBE (1:40 dilution) was subsequently added for 1hr at 37°C to allow formation of the terminal membrane complex. The cells were spun down and the OD₄₁₂ of the hemoglobin red supernatant was read to determine the extent of cell lysis.

[00132] Classical Pathway C5 Convertase Hemolytic Assay

[00133] The classical pathway C5 convertase hemolytic assay was run over a 2-day period. EshA (1×10^7 cells/tube) were shaken sequentially with 60SFU of human C1 (ART) for 15min, 60SFU of human C4 (Quidel) for 20min, and 10SFU human C2 (calculated after decay in a C3 convertase assay) (ART) along with 15SFU of human C3 (gift of C. Mold) for 5min, all in a 30°C water bath, to form the classical pathway C5 convertase C4b3b2a. Following this loading of complement components, 200ul DGVB⁺⁺ was added to each tube and the tubes were shaken for 2hr in a 30°C water bath to decay the C2. The cells were spun down, resuspended in DGVB⁺⁺, and decay continued overnight with the cells at 4°C. The

following day, the cells were shaken with 60SFU of human C1 for 15min in a 30°C water bath, followed by sufficient human C2 for a Z score of 1 (5min, 30°C). RCA proteins were added for 15min at 30°C to accelerate the decay of the C5 convertase. Subsequently, human C5 (Quidel) (1:250 dilution) was added for 5 min at 30°C, then guinea pig C6-9 in DGVBE (dextrose gelatin veronal buffer with EDTA) (1:150 dilution) for 1hr at 37°C. Cells were spun down and the OD₄₁₂ of the hemoglobin red supernatant was read to determine the extent of cell lysis.

[00134] Cofactor Experiments

[00135] The RCA proteins CR1, factor H and membrane cofactor protein MCP can act as cofactors for the factor I cleavage of C3b to smaller fragments (reviewed in M. Botto, "C3" [p. 88] in *The Complement FactsBook*, ed. B.J. Morley and M.J. Walport, 2000, San Diego: Academic Press). The cofactor activity of CR1 resides in its LHRs B and C (S.C. Makrides *et al.*, 1992, *J. Biol. Chem.* 267:24754; K.R. Kalli *et al.*, 1991, *J. Exp. Med.* 174:1451; M. Krych *et al.*, 1994, *J. Biol. Chem.* 269:13273). All three regulators can allow factor I to cleave C3b to iC3b. Factor I with CR1 can additionally cleave iC3b to C3c. When the disulfide bridges are reduced, an SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) gel will show the following bands:

C3b: α' (115kDa), β (75kDa)

iC3b: α' -68, α' -43, β (75kDa), and a small soluble fragment C3f

C3c: α' -27, α' -40, β (75kDa), and "membrane-attached" C3dg (40kDa)

(A. Sahu *et al.*, 1998, *J. Immunol.* 160:5596; A.M. Rosengard *et al.*, 2002 *PNAS* 99:8808).

[00136] To confirm that the hybrid retained the cofactor activity of CR1, human C3b (ART Lot 20P, 20ng), was mixed with human factor I (ART Lot 6P, 60ng). Factor H (500ng) or DAF - CR1BB (20ng) or a control of 10mM PO₄ buffer with 145mM NaCl, pH 7.3 was added for a total volume of 10ul and incubated for 19hr in a 37° water bath.

[00137] Samples of DAF-CRIB, DAF-CR1BB, and DAF-IgG4 separated on a 5% SDS polyacrylamide gel and were examined by Western blot using monoclonal antibodies

raised against DAF (IA10) or CR1 (E11) as shown in Fig. 14. Results indicated that the expressed hybrid proteins were recognized by antibodies to DAF or CR1 as expected.

[00138] A sample containing DAF-MCP was separated by 10% SDE PAGE and analyzed by Western blot using a monoclonal antibody raised against MCP (GB24) or DAF (IA 10), as shown in Fig. 15. The expressed DAF-MCP protein was recognized by GB 24 as expected.

[00139] Whole serum hemolytic assays were used to test the ability of the hybrid proteins to inhibit complement activity. Fig. 16 shows the percent inhibition of hemolysis of DAF - CR1BB and SCR1 control versus the concentration of the protein tested. 20-fold more SCR1 is required to achieve 50% inhibition than DAF-CR1BB. Fig. 17 shows the percent inhibition of hemolysis of DAF-MCP and DAF control versus the concentration of the protein tested. To achieve 25 percent inhibition, DAF requires more than 10 fold greater concentration than DAF-MCP.

[00140] The results of classical pathway C3 convertase hemolytic assays, using the hybrid proteins of the present invention are shown in Figs. 18 A and B, which are graphs showing the percent inhibition of hemolysis versus the concentration of proteins tested. Again, the hybrid protein containing a functional unit of DAF and two functional units of CR1 exhibited greater inhibition of hemolysis than protein containing only DAF CCPs 1-4.

[00141] The ability of the hybrid proteins to inhibit hemolysis in a classical pathway C5 convertase assay is shown in the graphs of Figs 19 and 20. In Fig 19, DAF - CR1B provides superior inhibition of hemolysis compared to DAF. In Fig. 20, DAF - CR1BB shows 19-fold greater inhibition of hemolysis than CR1 and 71-fold greater inhibition than DAF-CR1B.

[00142] The ability of DAF-MCP and DAF-CR1BB to act as cofactors for factor I is shown in Fig. 21. Samples were separated by SDS-PAGE, and developed with an anti-human C3 polyclonal antibody. Supernatants from COS cells were analyzed neat, with DAF - MCP or with DAF-CR1BB. Each of the samples was assayed with or without factor I (+I). Supernatants with factor I and either DAF-MCP or DAF-CR1BB both exhibited 43 and 40 kDa bands corresponding to formation of iC3b and C3c, respectively. The DAF-CR1BB sample additionally displayed a 29 kDa band corresponding to formation of C3c. A similar

result is shown in Fig. 22, where DAF-CR1B acts as cofactor for factor I as shown by the appearance of a band at 29 kDa.

[00143] Based upon the foregoing disclosure, it should be apparent that the present invention will carry out the aspects set forth above. It is therefore, to be understood that any variations evident fall within the scope of the invention and thus, the selection of specific component elements can be determined without departing from the spirit of the invention herein disclosed and described.

CLAIMS

We claim:

1. A protein comprising:

a first functional unit of a first complement regulatory protein, wherein the first functional unit exhibits complement-regulating properties;

a first spacer sequence of at least about 200 amino acids encoding a polypeptide that does not exhibit complement regulating properties, attached to the first functional unit; and

a second functional unit attached to the spacer sequence, selected from the group consisting of polypeptides providing a functional unit of a second complement regulatory protein, polypeptides derived from an immunoglobulin, and polypeptides that enhance binding of the protein to an animal cell.

2. The protein of claim 1, additionally comprising a second spacer sequence of at least about 200 amino acids encoding a polypeptide that does not exhibit complement regulating properties attached to the second function domain, and a third functional unit attached to the second spacer, wherein the third functional unit is selected from the group consisting of polypeptides derived from an immunoglobulin, and polypeptides that enhance binding of the protein to an animal cell.

3. The protein of claim 1, wherein the first functional unit comprises at least CCPs 2, 3 and 4 of DAF.

4. The protein of claim 1, wherein the second functional unit is selected from the group consisting of CCPs 8-10 of Complement Receptor 1 (CR1), CCPs 15-17 of CR1, CCPs 1-4 of Membrane Cofactor Protein (MCP), polypeptides derived from IgG4, and a lipid tail.
5. The protein of any of claims 1-4, wherein the spacers are selected from the group consisting of substantially all of the amino acids of CCPs 4-7 of CR1, and substantially all of the amino acids of CCPs 11-14 of CR1.
6. The protein of claim 1, wherein the first functional unit comprises CCPs 1, 2, 3 and 4 of DAF, the second functional unit is selected from the group consisting of CCPs 8-10 of CR1, CCPs 1-4 of Membrane Cofactor Protein (MCP), and polypeptides derived from IgG4, and the first spacer is substantially all of the amino acids of CCPs 4-7 of CR1.
7. The protein of claim 6, additionally comprising a second spacer comprising substantially all of the amino acids of CCPs 4-5 of CR1, and a third functional unit selected from the group consisting of CCPs 8-10 of CR1 CCPs 1-4 of MCP, and polypeptides derived from Ig G4.
8. A polynucleotide encoding the protein of claim 6.
9. A polynucleotide encoding the protein of claim 7.
10. A polynucleotide encoding the protein of claim 1.
11. A vector comprising the polynucleotide of claim 10.
12. A protein having at least 95 percent sequence homology to a protein selected from the group consisting of proteins having the sequence of SEQ. ID NO: 13, SEQ. ID NO: 15, SEQ. ID NO: 19, and SEQ. ID NO: 23.
13. A polynucleotide encoding the protein of claim 11.
14. A method of regulating complement activity comprising administering an effective amount of protein of claim 1 to a mammal.

15. The method of claim 15, wherein the mammal is a human.

Fig. 1A

SEQ. ID NO:1

MTVARPSVPAALPLLGELPRLLLLVLLCLPAVWGDCGLPPDVPNAQPALE 50
GRTSFPEDTVITYKCEESFVKIPGEKDSVICLKGSQWSDIEEFCNRSCEV 100
PTRLNSASLKQPYITQNYFPVGTVVEYECRPGYRREPSLSPKLTCLQNLK 150
WSTAVEFCKKKSCPNGEIRNGQIDVPGGILFGATISFSCNTGYKLFGST 200
SSFCLISGSSVQWSDPLPECREIYCPAPPQIDNGIIQGERDHYGYRQSVT 250
YACNKGFTMIGEHSIYCTVNNDEGEWSGPPPECRGKSLTSKVPPTVQKPT 300
TVNVPTTEVSPTSQKTTTKTTTPNAQATRSTPVSRTTKHFHETTPNKGSG 350
TTSGETRLLSGHTCFTLTGLLGLVTMGLLT

Fig. 1B

SEQ. ID NO:2

1 ccgctgggcg tagctgcgac tcggcggagt cccggcggcg cgtccttggt ctaaccgggc
61 gcgccatgac cgtcgcgcgg ccgagcgtgc ccgcggcgct gccctcctc ggggagctgc
121 cccggctgct gctgctggtg ctgttggtgc tgccggcgct gtggggtgac tgtggccttc
181 ccccagatgt acctaatgcc cagccagctt tggaaggccg tacaagtttt cccgaggata
241 ctgtaataac gtacaaatgt gaagaaagct ttgtgaaaat tcctggcgag aaggactcag
301 tgatctgcct taagggcagt caatggctcag atattgaaga gttctgcaat cgtagctgcg
361 aggtgccaac aaggctaaat tctgcatccc tcaaacagcc ttatatcact cagaattatt
421 ttccagtcgg tactgttggtg gaatatgagt gccgtccagg ttacagaaga gaaccttctc
481 tatcaccaaa actaacttgc cttcagaatt taaaatggtc cacagcagtc gaattttgta
541 aaaagaaatc atgccctaata cccggagaaa tacgaaatgg tcagattgat gtaccagggtg
601 gcatattatt tgggtgcaacc atctccttct catgtaacac agggtaacaa ttatttggtc
661 cgacttctag tttttgtctt atttcaggca gctctgtcca gtggagtgc ccgttgccag
721 agtgcagaga aatttattgt ccagcaccac cacaattga caatggaata attcaagggg
781 aacgtgacca ttatggatat agacagtctg taacgtatgc atgtaataaa ggattcacca
841 tgattggaga gcaactctatt tattgtactg tgaataatga tgaaggagag tggagtggcc
901 caccacctga atgcagagga aaatctctaa cttccaaggt cccaccaaca gttcagaaac
961 ctaccacagt aaatgttcca actacagaag tctcaccaac ttctcagaaa accaccacaa
1021 aaaccaccac accaaatgct caagcaacac ggagtacacc tgtttccagg acaaccaagc
1081 attttcatga aacaaccca aataaaggaa gtggaaccac ttcaggtact acccgtcttc
1141 tatctgggca cagtggtttc acgttgacag gtttgcttgg gacgctagta accatgggct
1201 tgctgactta gccaaagaag agttaagaag aaaatacaca caagtataca gactgttcct
1261 agtttcttag acttatctgc atattggata aaataaatgc aattgtgctc ttcatttagg
1321 atgctttcat tgtctttaag atgtgttagg aatgtcaaca gagcaaggag aaaaaaggca
1381 gtcctggaat cacattctta gcacacctac acctcttgaa aatagaacaa cttgcagaat
1441 tgagagtgat tcctttccta aaagtgtgaa aaagcataga gatttgttcg tatttagaat
1501 gggatcacga ggaaaagaga aggaaagtga tttttttcca caagatctgt aatgttattt
1561 ccacttataa aggaaataaa aaatgaaaaa cattatttgg atatcaaaag caaataaaaa
1621 cccaattcag tctcttctaa gcaaaattgc taaagagaga tgaaccacat tataaagtaa
1681 tctttggctg taaggcattt tcatctttcc ttcgggttgg caaaatattt taaaggtaaa
1741 acatgctggt gaaccagggg tggtgatggt gataaggagg gaatatagaa tgaaagactg
1801 aatcttcctt tgttgcaaaa atagagtttg gaaaaagcct gtgaaagggtg tcttctttga
1861 cttaatgtct ttaaaagtat ccagagatac tacaatatta acataagaaa agattatata
1921 ttatttctga atcgagatgt ccatagtc aaattgtaaat cttattcttt tgtaatatat
1981 atttatattt atttatgaca gtgaacattc tgattttaca tgtaaaacaa gaaaagttga
2041 agaagatatg tgaagaaaaa tgtatttttc ctaaatagaa ataaatgatc ccattttttg
2101 gt

Fig. 2

SEQ. ID NO:3

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KDRCCRKSCRNPPDPVNGMVHVIKGIQFGSQIKYSCTKGYRLIGSSSATC	150
IISGDTVWDNETPICDRIPCGLPPTITNGDFISTNRENFHYGSVVTYRC	200
NPGSGGRKVFELVGEPsiYCTSNDDQVGIWSGPAPQCIIPNKCTPPNVEN	250
GILVSDNRSLSLNEVVEFRCQPGFVMKGPRRVKCQALNKWEPELPSCSR	300
VCQPPPDVLHAERTQRDKDNFSPGQEVFYSCPGYDLRGAASMRCTPQGD	350
WSPAAPTCEVKSCDDFMGQLLNGRVLFVNLQLGAKVDFVCDEGFQLKGS	400
SASYCVLAGMESLWNSSVPVCEQIFCPSPPVIPNGRHTGKPLEVFPFGKA	450
VNYTCDPHPDRGTSFDLIGESTIRCTSDPQNGVWSSPAPRCGILGHCQA	500
PDHFLFAKLKTQTNASDFPIGTSLKYECRPEYYGRPFSITCLDNLVWSSP	550
KDVCKRKSKCTPPDPVNGMVHVIDIQVGSRIYNSCTTGHRIGHSSAEC	600
ILSGNAAHWSTKPPICQRIPCGLPPTIANGDFISTNRENFHYGSVVTYRC	650
NPGSGGRKVFELVGEPsiYCTSNDDQVGIWSGPAPQCIIPNKCTPPNVEN	700
GILVSDNRSLSLNEVVEFRCQPGFVMKGPRRVKCQALNKWEPELPSCSR	750
VCQPPPDVLHAERTQRDKDNFSPGQEVFYSCPGYDLRGAASMRCTPQGD	800
WSPAAPTCEVKSCDDFMGQLLNGRVLFVNLQLGAKVDFVCDEGFQLKGS	850
SASYCVLAGMESLWNSSVPVCEQIFCPSPPVIPNGRHTGKPLEVFPFGKA	900
VNYTCDPHPDRGTSFDLIGESTIRCTSDPQNGVWSSPAPRCGILGHCQA	950
PDHFLFAKLKTQTNASDFPIGTSLKYECRPEYYGRPFSITCLDNLVWSSP	1000
KDVCKRKSKCTPPDPVNGMVHVIDIQVGSRIYNSCTTGHRIGHSSAEC	1050
ILSGNTAHWSTKPPICQRIPCGLPPTIANGDFISTNRENFHYGSVVTYRC	1100
NLGSRGRKVFELVGEPsiYCTSNDDQVGIWSGPAPQCIIPNKCTPPNVEN	1150
GILVSDNRSLSLNEVVEFRCQPGFVMKGPRRVKCQALNKWEPELPSCSR	1200
VCQPPPEILHGEHTPSHQDNFSPGQEVFYSCPGYDLRGAASLHCTPQGD	1250
WSPEAPRCVAVKSCDDFLGQLPHGRVLFPLNLQLGAKVSFVCDEGFRLKGS	1300
SVSHCVLVGMRLWNNSVPVCEHIFCPNPPAILNGRHTGTSPGDIPIYGKE	1350
ISYTCDPHPDRGMTFNLIIGESTIRCTSDPHGNGVWSSPAPRCESVRAGH	1400
CKTPEQFPFASPTIPIINDFEFPVGTSNLNYECRPGYFGKMFSISCLNVLW	1450
SSVEDNCRKSCGPPPEPFNGMVHINTDTQFGSTVNYSNEGFRIGSPS	1500
TTCLVSGNNVTWDDKAPICEIISCEPPPTISNGDFYSNNRTSFHNGTVVT	1550
YQCHTGPDGEQLFELVGERSIYCTSKDDQVGWSSPPPRCISTNKCTAPE	1600
VENAIRVPGNRSFFSLTEIIRFRCQPGFVMVGSHTVQCQTNGRWGPKLPH	1650
CSRVCQPPPEILHGEHTLSHQDNFSPGQEVFYSCPSYDLRGAASLHCTP	1700
QGDWSPEAPRCTVKSCDDFLGQLPHGRVLLPLNLQLGAKVSFVCDEGFRL	1750
KGRSASHCVLAGMKALWNSSVPVCEQIFCPNPPAILNGRHTGTSPFGDIPY	1800
GKEISYACDTHPDRGMTFNLIIGESSIRCTSDPQNGVWSSPAPRCESLVP	1850
AACPHPPKIQNGHYIGGHVSLYLPGMTISYTCDPGYLLVGKGFIFCTDQG	1900
IWSQLDHYCKEVNCSFPLFMNGISKELEMKKVYHYGDYVTLKCEDGYTLE	1950
GSPWSQCQADDRWDPLAKCTSRADALIVGTLSGTIFFILLIIFLSWII	2000
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Fig. 3
SEQ. ID NO:4

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121 gtgctgcttg cgctgccggg ggcctggggg caatgcaatg ccccgagaatg gcttccattt
181 gccaggccta ccaacctaac tgatgagttt gagtttccca ttgggacata tctgaactat
241 gaatgccgcc ctggttattc cggaagaccg ttttctatca tctgcctaaa aaactcagtc
301 tggactgggtg ctaaggacag gtgcagacgt aaatcatgtc gtaatcctcc agatcctgtg
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421 actaaaggat accgactcat tggttcctcg tctgccacat gcatcatctc aggtgatact
481 gtcatttggg ataatgaaac acctatttgt gacagaattc cttgtgggct accccccacc
541 atcaccaatg gagatttcat tagcaccaac agagagaatt ttcactatgg atcagtgggtg
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661 tccatatact gcaccagcaa tgacgatcaa gtgggcatct ggagcggccc cgccctcag
721 tgcattatac ctaacaaatg cacgcctcca aatgtggaaa atggaatatt ggtatctgac
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 3361 tccatatact gcaccagcaa tgacgatcaa gtgggcatct ggagcggccc cgccctcag
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Fig. 4A

SEQ. ID NO:5

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SGKPPICEKVLCTPPPPIKNGKHTFSEVEVFEYLDVAVTYSCDPAPGDPF 200
SLIGESTIYCGDNSVWSRAAPECKVVKCRFPVVENGKQISGFGKKFYKA 250
TVMFECDKGFYLDGSDTIVCDSNSTWDPPVPKCLKVSTSSTTKSPASSAS 300
GPRPTYKPPVSNYPGYPKPEEGILDSLDVWVIAVIVIAIVVGVAVICVVP 350
YRYLQRRKKKGKADGGAETATYQTKSTTPAEQRG
```

Fig. 4B

SEQ. ID NO:6

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721 caaatgtcga tttccagtag tcgaaaatgg aaaacagata tcaggatttg gaaaaaatt
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1501 tagttcacia tgaaattata ttttctttgt
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Fig. 5

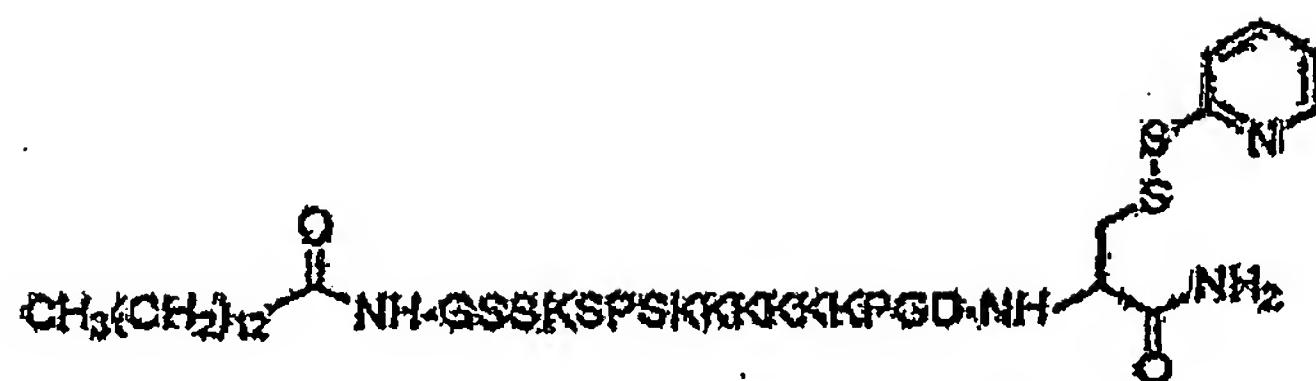


Fig. 6A

SEQ. ID NO:7

ATA TAC GAA TTC AGA TCT ATG ACC GTC GCG CGG CCG AGC GTG

Fig. 6B

SEQ. ID NO:8

ACA GTG CTC GAG CAT TCA GGT GGT GGG CCA CTC CA

Fig 7A

SEQ. ID NO:9

ATA TAC CTC GAG TCC TAA CAA ATG CAC GCC TCC AAA TGT GG-3

Fig 7B

SEQ. ID NO:10

ACA GTG ATG CAT TGG TTT GGG TTT TCA ACT TGG C

Fig 7C

SEQ. ID NO:11

ATA TAC ATG CAT CTG ACT TTC CCA TTG GGA CAT CTT TAA AG

Fig 7D

SEQ. ID NO:12

ACA GTG AGA TCT TTA GTG ATG GTG ATG GTG ATG AAT TCC ACA GCG AGG GGC
AGG GCT

Fig. 8A
SEQ ID NO:13

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D S V P C L R G S Q W S D I E K E F C N R S
C E V P T R V N S A S L K Q P Y R I T E Q N Y
F P V G T V L E Y E C R P G Y R R E F C S L
S P K L T C P G E I R N G Q I D V P G G I K
K S C P T N P S S C N T G Y K L F G S T S L
F G A T I S G S V Q W S D P L F P E C R E I
F C L I S G S I D N G I I Q G E R C D H Y G
Y C P A P P Q Y A C N K G I F T M I G E H S I
Y C T V N N D E G E W S G P P I L V C D N
S S P N K C T P V V E F R C Q P G F V M
R S L F R R V K C Q P P D V L N K W E P G L P S
K G P R R C Q P P D V L N K W E P G L P S
C S R V C P G Q P P D V L N K W E P G L P S
K D N F S P G Q P P D V L N K W E P G L P S
R G A A S M R C T P Q G D W S P G A V L T
C E V K S C D D F M G Q L L N G E R A V L F
P V N S A S Y C V L A G M E S L W N S S
K G S S A S Y C V L A G M E S L W N S S
V P V C E Q I F C P S P P V I P N G R H
T G K P L E V F P F G K A V N Y T C D P
H P D R G T S F D L I G A P R C I R C T S
D P Q G N G V W S S P A K L K T Q Y Y G R P F
C Q A P D H F L Y E C R P E Y Y G R P F
F P I G T S L K V W S M V H V I T D I Q
S I T C P P D P V N G M V H V I T D I Q
S C K T P P D P V N G M V H V I T D I Q
V G S R I N Y S C T T G H R L I G H S S
A E C I L S G N A A H W S T K P P I C Q
R I P C G L P P T I A N G D F I S T N R
E N F H Y G S V V T Y R C N P G S G G R
K V F E L V G E P S I I C N S D D Q V
G I W S G P A P Q C I I P N K C T P P N
V E N G I L V S D N R S L F S L N E V V
E F R C Q P G F V M K G P R V C Q P P D
L N K W E P T Q R D K S R V C Q P P D
V L H A E R T Q R D K S R V C Q P P D
F Y S C E P G Y D L R G A A S M R C D F M
Q G Q L L N G R V L F P K G S S A S C D A K V
D F V C D E L G W N G F Q L S V P V C E Q I V C P F
A G M E S L P N G R S H T G P K P L E V F C P F
S P P V I P N G R S H T G P K P L E V F C P F
G K A V N Y T C D P H P D R G T S F D L

I G E S T I R C T S D P Q G N G V W S S
P A P R C G I H H H H H H

Fig. 8B

SEQ. ID NO: 14

ATGACCGTTCGCGCGGCCGAGCGTGCCCGCGGCGCTGCCCTCCTCGGGGAGCTGCCCCGGCTGCTGCTGCTGGTGCTGTTGTGCCTGCCGGCCGTGTGGGGTGACTGTGGCCTTCCCCCAGATGTACCTAATGCCCAGCCAGCTTTGGAAGGCCGTACAAGTTTTCCCGAGGATACTGTAATAACGTACAAATGTGAAGAAAGCTTTGTGAAAATTCTGGCGAGAAGGACTCAGTGATCTGCCTTAAGGGCAGTCAATGGTCAGATATTGAAGAGTTCTGCAATCGTAGCTGCGAGGTGCCAACAAAGGCTAAATTCTGCATCCCTCAAACAGCCTTATATCACTCAGAATTATTTCCAGTCGGTACTGTTGTGGAATATGAGTGCCGTCAGGTTACAGAAGAGAACCTTCTCTATCACCAAAACTAACTTGCCTTCAGAATTTAAAATGGTCCACAGCAGTCGAATTTTGTAAAAAGAAATCATGCCCTAATCCGGGAGAAATACGAAATGGTCAGATTGATGTACCAGGTGGCATATTA

TTTGGTGCAACCATCTCCTTCTCATGTAACACAGGGTACAAATTATTTGGCTCGACTTCTAGTTTTTTGTCTTATTTTCAGGCAGCTCTGTCCAGTGAGTGACCCGTTGCCAGAGTGACAGAGAAATTATTGTCCAGCACCAACCAAATTGACAATGGAATAATTCAAGGGGAACGTGACCATATATGGATATAGACAGTCTGTAACGTATGCATGTAATAAAGGATTCACCATGATTGGAGAGCACTCTATTATTGTACTGTGAATAATGATGAAGGAGAGTGAGTGGCCACCACCTGAATGC

TCGAGTCCTAACAAATGCACGCCTCCAAATGTGGAAAATGGAATATTGGTATCTGACAACAGAAGCTTATTTTCTTAAATGAAGTTGTGGAGTTAGGTGTCAGCCTGGCTTTGTCTATGAAAGGACCCCGCCGTGTGAAGTGCCAGGCCCTGAACAAATGGGAGCCGGAGCTACCAAGCTGCTCCAGGGTATGTCAGCCACCTCCAGATGTCTGTCATGCTGAGCGTACCCAAAGGGACAAGGACAACCTTTTACCTGGGCAGGAAGTGTCTACAGCTGTGAGCCCGGCTACGACCTCAGAGGGGCTGCGTCTATGCGCTGCACACCCAGGGAGACTGGAGCCCTGCAGCCCCCACA

TGTGAAGTGAAATCCTGTGATGACTTCATGGGCCAACTTCTTAATGGCCGTGTGCTATTTCCAGTAAATCTCCAGCTTGGAGCAAAAGTGGATTTTGTTTGTGATGAAGGATTTCAATTA

AAAGGCAGCTCTGCTAGTTACTGTGTCTTGGCTGGAATGGAAAGCCTTTGGAATAGCAGTGTTCCAGTGTGTGAACAAATCTTTTGTCCAAGTCTCCAGTTATTCCTAATGGGAGACACACAGGAAAACCTCTGGAAGTCTTTCCCTTTGGAAAAGCAGTAAATTACACATGCGACCCC

CACCCAGACAGAGGGACGAGCTTCGACCTCATTTGGAGAGAGCACCATCCGCTGCACAAGTGACCTCAAGGGAATGGGGTTTGGAGCAGCCCTGCCCTCGCTGTGGAATTCTGGGTAC

TGTCAAGCCCCAGATCATTTTCTGTTTGGCCAAGTTGAAAACCCAAACCAATGCATCTGACTTTCCCATTTGGGACATCTTTAAAGTACGAATGCCGTCTGAGTACTACGGGAGGCCATTCTATACATGTCTAGATAACCTGGTCTGGTCAAGTCCCAAAGATGTCTGTAAACGTAAATCATGTAAAACCTCCTCCAGATCCAGTGAATGGCATGGTGCATGTGATCACAGACATCCAG

GTTGGATCCAGAATCAACTATTCTTGTACTACAGGGCACCGACTCATTGGTCACTCATCTGCTGAATGTATCCTCTCGGGCAATGCTGCCCATTTGGAGCACGAAGCCGCCAATTTGTCAACGAATTCCTTGTGGGCTACCCCCCACCATCGCCAATGGAGATTTCAATTAGCACCAACAGAGAGAATTTTCACTATGGATCAGTGGTGACCTACCGCTGCAATCCTGGAAGCGGAGGGAGA

AAGGTGTTTGTGAGCTTGTGGGTGAGCCCTCCATATACTGCACCAGCAATGACGATCAAGTGGCATCTGGAGCGGCCCGGCCCTCAGTGCAATTATACCTAACAAATGCACGCCTCCAAATGTGGAAAATGGAATATTGGTATCTGACAACAGAAGCTTATTTTCTTAAATGAAGTTGTG

GAGTTTAGGTGTGAGCCTGGCTTTGTGTCATGAAAGGACCCCGCCGTGTGAAGTGCCAGGCCCTGAACAAATGGGAGCCGGAGCTACCAAGCTGCTCCAGGGTATGTGAGCCACCTCCAGATGTCTGTCATGCTGAGCGTACCCAAAGGGACAAGGACAACCTTTTACCCGGGCAGGAAGTGTCTACAGCTGTGAGCCCGGCTATGACCTCAGAGGGGCTGCGTCTATGCGCTGCACACCC

CAGGGAGACTGGAGCCCTGCAGCCCCCACATGTGAAGTGAAATCCTGTGATGACTTCATGGGCCAACTTCTTAATGGCCGTGTGCTATTTCCAGTAAATCTCCAGCTTGGAGCAAAAGTG

GATTTTGTGTTGTGATGAAGGATTTCAATTAAAAGGCAGCTCTGCTAGTTATTGTGTCTTG
GCTGGAATGGAAAGCCTTTTGAATAGCAGTGTTCAGTGTGTGAACAAATCTTTTGTCCA
AGTCCTCCAGTTATTCTAATGGGAGACACACAGGAAAACCTCTGGAAGTCTTTCCCTTT
GGAAAAGCAGTAAATTACACATGCGACCCCCACCCAGACAGAGGGACGAGCTTCGACCTC
ATTGGAGAGAGCACCATCCGCTGCACAAGTGACCCTCAAGGGAATGGGGTTTGGAGCAGC
CCTGCCCCTCGCTGTGGAATTCATCACCATCACCATCACTAAAGATCT

Fig. 9A
SEQ ID NO:15

	L	L	G	E	L	P	R	M	T	V	A	R	P	S	V	P	A	A	L	P	
	L	L	G	E	L	P	R	L	L	L	L	V	L	L	C	L	P	A	V	W	G
D	C	G	L	P	P	D	V	P	N	A	Q	P	A	L	E	G	R	T	S	F	
P	E	D	T	V	I	T	Y	K	C	E	E	S	F	V	K	I	P	G	E	K	
D	S	V	I	C	L	K	G	S	Q	W	S	D	I	E	E	F	C	N	R	S	
C	E	V	P	T	R	L	N	S	A	S	L	K	Q	P	Y	I	T	Q	N	Y	
F	P	V	G	T	V	V	E	Y	E	C	R	P	G	Y	R	R	E	P	S	L	
S	P	K	L	T	C	L	Q	N	L	K	W	S	T	A	V	E	F	C	K	K	
K	S	C	P	N	P	G	E	I	R	N	G	Q	I	D	V	P	G	G	I	L	
F	G	A	T	I	S	F	S	C	N	T	G	Y	K	L	F	G	S	T	S	S	
F	C	L	I	S	G	S	S	V	Q	W	S	D	P	L	P	E	C	R	E	I	
Y	C	P	A	P	P	Q	I	D	N	G	I	I	Q	G	E	R	D	H	Y	G	
Y	R	Q	S	V	T	Y	A	C	N	K	G	F	T	M	I	G	E	H	S	I	
Y	C	T	V	N	N	D	E	G	E	W	S	G	P	P	P	E	C				
S	S	P	N	K	C	T	P	P	N	V	E	N	G	I	L	V	S	D	N		
R	S	L	F	S	L	N	E	V	V	E	F	R	C	Q	P	G	F	V	M		
K	G	P	R	R	V	K	C	Q	A	L	N	K	W	E	P	E	L	P	S		
C	S	R	V	C	Q	P	P	P	D	V	L	H	A	E	R	T	Q	R	D		
K	D	N	F	S	P	G	Q	E	V	F	Y	S	C	E	P	G	Y	D	L		
R	G	A	A	S	M	R	C	T	P	Q	G	D	W	S	P	A	A	P	T		
C	E	V	K	S	C	D	D	F	M	G	Q	L	L	N	G	R	V	L	F		
P	V	N	L	Q	L	G	A	K	V	D	F	V	C	D	E	G	F	Q	L		
K	G	S	S	A	S	Y	C	V	L	A	G	M	E	S	L	W	N	S	S		
V	P	V	C	E	Q	I	F	C	P	S	P	P	V	I	P	N	G	R	H		
T	G	K	P	L	E	V	F	P	F	G	K	A	V	N	Y	T	C	D	P		
H	P	D	R	G	T	S	F	D	L	I	G	E	S	T	I	R	C	T	S		
D	P	Q	G	N	G	V	W	S	S	P	A	P	R	C	G	I	L	G	H		
C	Q	A	P	D	H	F	L	F	A	K	L	K	T	Q	T	N	A	S	D		
F	P	I	G	T	S	L	K	Y	E	C	R	P	E	Y	Y	G	R	P	F		
S	I	T	C	L	D	N	L	V	W	S	S	P	K	D	V	C	K	R	K		
S	C	K	T	P	P	D	P	V	N	G	M	V	H	V	I	T	D	I	Q		
V	G	S	R	I	N	Y	S	C	T	T	G	H	R	L	I	G	H	S	S		
A	E	C	I	L	S	G	N	A	A	H	W	S	T	K	P	P	I	C	Q		
R	I	P	C	G	L	P	P	T	I	A	N	G	D	F	I	S	T	N	R		
E	N	F	H	Y	G	S	V	V	T	Y	R	C	N	P	G	S	G	G	R		
K	V	F	E	L	V	G	E	P	S	I	Y	C	T	S	N	D	D	Q	V		
G	I	W	S	G	P	A	P	Q	C	I	I	P	N	K	C	T	P	P	N		

V E N G I L V S D N R S L F S L N E V V
E F R C Q P G F V M K G P R R V K C Q A
L N K W E P E L P S C S R V C Q P P D
V L H A E R T Q R D K D N F S P G Q E V
F Y S C E P G Y D L R G A A S M R C T P
Q G D W S P A A P T C E V K S C D D F M
G Q L L N G R V L F P V N L Q L G A K V
D F V C D E G F Q L K G S S A S Y C V L
A G M E S L W N S S V P V C E Q I F C P
S P P V I P N G R H T G K P L E V F P F
G K A V N Y T C D P H P D R G T S F D L
I G E S T I R C T S D P Q G N G V W S S
P A P R C G I L G H C Q A P D H F L F A
K L K T Q T N A S D F P I G T S L K Y E
C R P E Y Y G R P F S I T C L D N L V W
S S P K D V C K R K S C K T P P D P V N
G M V H V I T D I Q V
G S R I N Y S C T T G H R L I G H S S
A E C I L S G N A A H W S T K P P I C Q
R I P C G L P P T I A N G D F I S T N R
E N F H Y G S V V T Y R C N P G S G G R
K V F E L V G E P S I Y C T S N D D Q V
G I W S G P A P Q C I I P N K C T P P N
V E N G I L V S D N R S L F S L N E V V
E F R C Q P G F V M K G P R R V K C Q A
L N K W E P E L P S C S R V C Q P P D
V L H A E R T Q R D K D N F S P G Q E V
F Y S C E P G Y D L R G A A S M R C T P
Q G D W S P A A P T C E V K S C D D F M
G Q L L N G R V L F P V N L Q L G A K V
D F V C D E G F Q L K G S S A S Y C V L
A G M E S L W N S S V P V C E Q I F C P
S P P V I P N G R H T G K P L E V F P F
G K A V N Y T C D P H P D R G T S F D L
I G E S T I R C T S D P Q G N G V W S S
P A P R C G I H H H H H H H

Fig 9B

SEQ. ID NO:16

ATGACCGTCGCGCGGCCGAGCGTGCCCGCGGCGCTGCCC
CTCCTCGGGGAGCTGCCCCGGCTGCTGCTGCTGGTGCTGTTGTGCCTGCCGGCCGTGTGGGGT
GACTGTGGCCTTCCCCCAGATGTACCTAATGCCAGCCAGCTTTGGAAGGCCGTACAAGTTTT
CCCGAGGATACTGTAATAACGTACAAATGTGAAGAAAGCTTTGTGAAAATTCCTGGCGAGAAG
GACTCAGTGATCTGCCTTAAGGGCAGTCAATGGTCAGATATTGAAGAGTTCTGCAATCGTAGC
TGCGAGGTGCCAACAAGGCTAAATTCTGCATCCCTCAAACAGCCTTATATCACTCAGAATTAT
TTTCCAGTCGGTACTGTTGTGGAATATGAGTGCCGTCCAGGTTACAGAAGAGAACCTTCTCTA

TCACCAAACTAACTTGCCTTCAGAATTTAAAATGGTCCACAGCAGTCGAATTTTGTA AAAAG
AAATCATGCCCTAATCCGGGAGAAATACGAAATGGTCAGATTGATGTACCAGGTGGCATATTA
TTTGGTGCAACCATCTCCTTCTCATGTAACACAGGGTACAAATTATTTGGCTCGACTTCTAGT
TTTTGTCTTATTTTCAGGCAGCTCTGTCCAGTGAGTGACCCGTTGCCAGAGTGCAGAGAAATT
TATTGTCCAGCACCACCACAAATTGACAATGGAATAATTCAAGGGGAACGTGACCATTATGGA
TATAGACAGTCTGTAACGTATGCATGTAATAAAGGATTCACCATGATTGGAGAGCACTCTATT
TATTGTACTGTGAATAATGATGAAGGAGAGTGGAGTGGCCCAACCACTGAATGC
TCGAGTCCTAACAAATGCACGCCTCCAAATGTGGAAAATGGAATATTGGTATCTGACAAC
AGAAGCTTATTTTCCTTAAATGAAGTTGTGGAGTTTAGGTGTCAGCCTGGCTTTGTCATG
AAAGGACCCCGCCGTGTGAAGTGCCAGGCCCTGAACAAATGGGAGCCGGAGCTACCAAGC
TGCTCCAGGGTATGTCAGCCACCTCCAGATGTCCTGCATGCTGAGCGTACCCAAAGGGAC
AAGGACAACCTTTTCACCTGGGCAGGAAGTGTTCTACAGCTGTGAGCCCGGCTACGACCTC
AGAGGGGCTGCGTCTATGCGCTGCACACCCCAAGGAGACTGGAGCCCTGCAGCCCCCACA
TGTGAAGTGAAATCCTGTGATGACTTCATGGGCCAACTTCTTAATGGCCGTGTGCTATTT
CCAGTAAATCTCCAGCTTGGAGCAAAAGTGGATTTTGTGTTGTGATGAAGGATTTCAATTA
AAAGGCAGCTCTGCTAGTTACTGTGCTTGGCTGGAATGGAAAGCCTTTGGAATAGCAGT
GTTCCAGTGTGTGAACAAATCTTTTGTCCAAGTCTCCAGTTATTCCTAATGGGAGACAC
ACAGGAAAACCTCTGGAAGTCTTTCCCTTTGGAAAAGCAGTAAATTACACATGCGACCCC
CACCCAGACAGAGGGACGAGCTTCGACCTCATTTGGAGAGAGCACCATCCGCTGCACAAGT
GACCCTCAAGGGAATGGGGTTTGGAGCAGCCCTGCCCTCGCTGTGGAATTCCTGGGTCAC
TGTCAGCCCCAGATCATTCTGTTTGCCAAGTTGAAAACCCAAACCAATGCATCTGAC
TTTCCCATTGGGACATCTTTAAAGTACGAATGCCGTCTGAGTACTACGGGAGGCCATTC
TCTATCACATGTCTAGATAACCTGGTCTGGTCAAGTCCCAAAGATGTCTGTAAACGTAAA
TCATGTAAACTCCTCCAGATCCAGTGAATGGCATGGTGCATGTGATCACAGACATCCAG
GTTGGATCCAGAATCAACTATTCTTGTAACAGGGCACCGACTCATTGGTCACTCATCT
GCTGAATGTATCCTCTCGGGCAATGCTGCCCATTTGGAGCACGAAGCCGCCAATTTGTCAA
CGAATTCCTTGTGGGCTACCCCCCACCATCGCCAATGGAGATTTCAATTAGCACCAACAGA
GAGAATTTTCACTATGGATCAGTGGTGACCTACCGCTGCAATCCTGGAAGCGGAGGGAGA
AAGGTGTTTGAGCTTGTGGGTGAGCCCTCCATATACTGCACCAGCAATGACGATCAAGTG
GGCATCTGGAGCGGCCCCGGCCCCCTCAGTGCATTATACCTAACAAATGCACGCCTCCAAAT
GTGGAAAATGGAATATTGGTATCTGACAACAGAAGCTTATTTTCCTTAAATGAAGTTGTG
GAGTTTAGGTGTCAGCCTGGCTTTGTCATGAAAGGACCCCGCCGTGTGAAGTGCCAGGCC
CTGAACAAATGGGAGCCGGAGCTACCAAGCTGCTCCAGGGTATGTCAGCCACCTCCAGAT
GTCCTGCATGCTGAGCGTACCCAAAGGGACAAGGACAACCTTTTCACCCGGGCAGGAAGTG
TTCTACAGCTGTGAGCCCGGCTATGACCTCAGAGGGGCTGCGTCTATGCGCTGCACACCC
CAGGGAGACTGGAGCCCTGCAGCCCCCAGATGTGAAGTGAAATCCTGTGATGACTTCATG
GGCCAACCTTCTTAATGGCCGTGTGCTATTTCCAGTAAATCTCCAGCTTGGAGCAAAAGTG
GATTTTGTGTTGTGATGAAGGATTTCAATTAAAAGGCAGCTCTGCTAGTTATTGTGTCTTG
GCTGGAATGGAAAGCCTTTGGAATAGCAGTGTTCAGTGTGTGAACAAATCTTTTGTCCA
AGTCCTCCAGTTATTCCTAATGGGAGACACACAGGAAAACCTCTGGAAGTCTTTCCCTTT
GGAAAAGCAGTAAATTACACATGCGACCCCCACCCAGACAGAGGGACGAGCTTCGACCTC
ATTGGAGAGAGCACCATCCGCTGCACAAGTGACCCTCAAGGGAATGGGGTTTGGAGCAGC
CCTGCCCCCTCGCTGTGGAATCTGGGTCACTGTCAAGCCCCAGATCATTCTGTGTTGCC
AAGTTGAAAACCCAAACCAATGCATCTGACTTTCCCATTTGGGACATCTTTAAAGTACGAA
TGCCGTCCTGAGTACTACGGGAGGCCATTCTCTATCACATGTCTAGATAACCTGGTCTGG
TCAAGTCCCAAAGATGTCTGTAAACGTAAATCATGTAAACTCCTCCAGATCCAGTGAAT
GGCATGGTGCATGTGATCACAGACATCCAGGT
GGATCCAGAATCAACTATTCTTGTAACAGGGCACCGACTCATTGGTCACTCATCT
GCTGAATGTATCCTCTCGGGCAATGCTGCCCATTTGGAGCACGAAGCCGCCAATTTGTCAA
CGAATTCCTTGTGGGCTACCCCCCACCATCGCCAATGGAGATTTCAATTAGCACCAACAGA
GAGAATTTTCACTATGGATCAGTGGTGACCTACCGCTGCAATCCTGGAAGCGGAGGGAGA

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AAGGTGTTTGAGCTTGTGGGTGAGCCCTCCATATACTGCACCAGCAATGACGATCAAGTG
GGCATCTGGAGCGGCCCGGCCCTCAGTGCATTATACCTAACAAATGCACGCCTCCAAAT
GTGGAAAATGGAATATTGGTATCTGACAACAGAAGCTTATTTTCTTAAATGAAGTTGTG
GAGTTTAGGTGTCAGCCTGGCTTTGTTCATGAAAGGACCCCGCGTGTGAAGTGCCAGGCC
CTGAACAAATGGGAGCCGGAGCTACCAAGCTGCTCCAGGGTATGTCAGCCACCTCCAGAT
GTCCTGCATGCTGAGCGTACCCAAAGGGACAAGGACAACCTTTTCACCCGGGCAGGAAGTG
TTCTACAGCTGTGAGCCCGCTATGACCTCAGAGGGGCTGCGTCTATGCGCTGCACACCC
CAGGGAGACTGGAGCCCTGCAGCCCCACATGTGAAGTGAAATCCTGTGATGACTTCATG
GGCCAACTTCTTAATGGCCGTGTGCTATTTCCAGTAAATCTCCAGCTTGGAGCAAAGTG
GATTTTGTGGTGTGATGAAGGATTTCAATTAAAAGGCAGCTCTGCTAGTTATTGTGTCTTG
GCTGGAATGGAAAGCCTTTGGAATAGCAGTGTTCCAGTGTGTGAACAAATCTTTTGTCCA
AGTCCTCCAGTTATTCCTAATGGGAGACACACAGGAAAACCTCTGGAAGTCTTTCCCTTT
GGAAAAGCAGTAAATTACACATGCGACCCCCACCCAGACAGAGGGACGAGCTTCGACCTC
ATTGGAGAGAGCACCATCCGCTGCACAAGTGACCTCAAGGGAATGGGGTTTGGAGCAGC
CCTGCCCTCGCTGTGGAATTCATCACCATCACCATCACTAAAGATCT

Fig. 10A

SEQ. ID NO:17

ATA TAC GAA TTC TGG TTG AGT CCA AAT ATG GTC CC

Fig. 10B

SEQ. ID NO:18

ACA GTG AGA TCT TTA TCA TTT ACC CGG AGA CAG GGA G

Fig. 11A

SEQ. ID NO:19

M T V A R P S V P A A L P																				
L	L	G	E	L	P	R	L	L	L	L	V	L	L	C	L	P	A	V	W	G
D	C	G	L	P	P	D	V	P	N	A	Q	P	A	L	E	G	R	T	S	F
P	E	D	T	V	I	T	Y	K	C	E	E	S	F	V	K	I	P	G	E	K
D	S	V	I	C	L	K	G	S	Q	W	S	D	I	E	E	F	C	N	R	S
C	E	V	P	T	R	L	N	S	A	S	L	K	Q	P	Y	I	T	Q	N	Y
F	P	V	G	T	V	V	E	Y	E	C	R	P	G	Y	R	R	E	P	S	L
S	P	K	L	T	C	L	Q	N	L	K	W	S	T	A	V	E	F	C	K	K
K	S	C	P	N	P	G	E	I	R	N	G	Q	I	D	V	P	G	G	I	L
F	G	A	T	I	S	F	S	C	N	T	G	Y	K	L	F	G	S	T	S	S
F	C	L	I	S	G	S	S	V	Q	W	S	D	P	L	P	E	C	R	E	I
Y	C	P	A	P	P	Q	I	D	N	G	I	I	Q	G	E	R	D	H	Y	G
Y	R	Q	S	V	T	Y	A	C	N	K	G	F	T	M	I	G	E	H	S	I
Y	C	T	V	N	N	D	E	G	E	W	S	G	P	P	P	E	C			
S	S	P	N	K	C	T	P	P	N	V	E	N	G	I	L	V	S	D	N	

R S L F S L N E V V E F R C Q P G F V M
K G P R R V K C Q A L N K W E P E L P S
C S R V C Q P P P D V L H A E R T Q R D
K D N F S P G Q E V F Y S C E P G Y D L
R G A A S M R C T P Q G D W S P A A P T
C E V K S C D D F M G Q L L N G R V L F
P V N L Q L G A K V D F V C D E G F Q L
K G S S A S Y C V L A G M E S L W N S S
V P V C E Q I F C P S P P V I P N G R H
T G K P L E V F P F G K A V N Y T C D P
H P D R G T S F D L I G E S T I R C T S
D P Q G N G V W S S P A P R C G I L
V E S K Y G P P C P S C P A P E F L
G G P S V F L F P P K P K D T L M I S R
T P E V T C V V V D V S Q E D P E V Q F
N W Y V D G V E V H N A K T K P R E E Q
F N S T Y R V V S V L T V L H Q D W L N
G K E Y K C K V S N K G L P S S I E K T
I S K A K G Q P R E P Q V Y T L P P S Q
E E M T K N Q V S L T C L V K G F Y P S
D I A V E W E S N G Q P E D N Y K T T P
P V L D S D G S F F L Y S R L T V D K S
R W Q E G N V F S C S V M H E A L H N H
Y T Q K S L S L S P G K

Fig. 11B
SEQ. ID NO:20

ATGACCGTCGCGCGGCCGAGCGTGCCCCGCGCGCTGCCC
CTCCTCGGGGAGCTGCCCCGGCTGCTGCTGCTGGTGCTGTTGTGCCTGCCGGCCGTGTGGGGT
GACTGTGGCCTTCCCCCAGATGTACCTAATGCCCAGCCAGCTTTGGAAGGCCGTACAAGTTTT
CCCGAGGATACTGTAATAACGTACAAATGTGAAGAAAGCTTTGTGAAAATTCCTGGCGAGAAG
GACTCAGTGATCTGCCTTAAGGGCAGTCAATGGTCAGATATTGAAGAGTTCTGCAATCGTAGC
TGCGAGGTGCCAACAAGGCTAAATTCTGCATCCCTCAAACAGCCTTATATCACTCAGAATTAT
TTTCCAGTCGGTACTGTTGTGGAATATGAGTGCCGTCCAGGTTACAGAAGAGAACCTTCTCTA
TCACCAAACTAACTTGCCTTCAGAATTTAAAATGGTCCACAGCAGTCGAATTTTGTA AAAAG
AAATCATGCCCTAATCCGGGAGAAATACGAAATGGTCAGATTGATGTACCAGGTGGCATATTA
TTTGGTGCAACCATCTCCTTCTCATGTAACACAGGGTACAAATTATTTGGCTCGACTTCTAGT
TTTTGTCTTATTTT CAGGCAGCTCTGTCCAGTGAGTGACCCGTTGCCAGAGTGCAGAGAAATT
TATTGTCCAGCACCACCACAAATTGACAATGGAATAATTCAAGGGGAACGTGACCATTATGGA
TATAGACAGTCTGTAACGTATGCATGTAATAAAGGATTACCATGATTGGAGAGCACTCTATT
TATTGTACTGTGAATAATGATGAAGGAGAGTGAGTG GCCCACCACTGAATGC
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AAAGGCAGCTCTGCTAGTTACTGTGTCTTGGCTGGAATGGAAAGCCTTTGGAATAGCAGT
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ACAGGAAAACCTCTGGAAGTCTTTCCCTTTGGAAAAGCAGTAAATTACACATGCGACCCC
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GACCCTCAAGGGAATGGGGTTTGGAGCAGCCCTGCCCTCGCTGTGGAATTCTG
GTTGAGTCCAAATATGGTCCCCCATGCCCATCATGCCCAGCACCTGAGTTCCTG
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ACCCCTGAGGTACGTCGTCGTGGTGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTC
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GGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCTTCATCGAGAAAACC
ATCTCCAAAGCCAAAGGGCAGCCCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAG
GAGGAGATGACCAAGAACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGC
GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGGACAAC TACAAGACCACGCCT
CCCGTGTCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAGGCTAACCGTGGACAAGAGC
AGGTGGCAGGAGGGGAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAC
TACACACAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGATAAAGATCT

Fig. 12A

SEQ. ID NO:21

ATA TAC GAA TTC TGG GTC ACT GTG AGG AGC CAC CAA CAT TTG AAG C

Fig. 12B

SEQ. ID NO:22

ACA GTG AGA TCT TTA GTG ATG GTG ATG GTG ATG CGA CAC TTT AAG ACA CTT
TGG AAC

Fig. 13A

SEQ. ID NO:23

M	T	V	A	R	P	S	V	P	A	A	L	P							
L	L	G	E	L	P	R	L	L	L	V	L	L	C	L	P	A	V	W	G
D	C	G	L	P	P	D	V	P	N	A	Q	P	A	L	E	G	R	T	S
P	E	D	T	V	I	T	Y	K	C	E	E	S	F	V	K	I	P	G	E
D	S	V	I	C	L	K	G	S	Q	W	S	D	I	E	E	F	C	N	R
C	E	V	P	T	R	L	N	S	A	S	L	K	Q	P	Y	I	T	Q	N
F	P	V	G	T	V	V	E	Y	E	C	R	P	G	Y	R	R	E	P	S
S	P	K	L	T	C	L	Q	N	L	K	W	S	T	A	V	E	F	C	K
K	S	C	P	N	P	G	E	I	R	N	G	Q	I	D	V	P	G	G	I
F	G	A	T	I	S	F	S	C	N	T	G	Y	K	L	F	G	S	T	S
F	C	L	I	S	G	S	S	V	Q	W	S	D	P	L	P	E	C	R	E

Y C P A P P Q I D N G I I Q G E R D H Y G
Y R Q S V T Y A C N K G F T M I G E H S I
Y C T V N N D E G E W S G P P P E C
S S P N K C T P P N V E N G I L V S D N
R S L F S L N E V V E F R C Q P G F V M
K G P R R V K C Q A L N K W E P E L P S
C S R V C Q P P P D V L H A E R T Q R D
K D N F S P G Q E V F Y S C E P G Y D L
R G A A S M R C T P Q G D W S P A A P T
C E V K S C D D F M G Q L L N G R V L F
P V N L Q L G A K V D F V C D E G F Q L
K G S S A S Y C V L A G M E S L W N S S
V P V C E Q I F C P S P P V I P N G R H
T G K P L E V F P F G K A V N Y T C D P
H P D R G T S F D L I G E S T I R C T S
D P Q G N G V W S S P A P R C G I L G H
C E E P P T F E A M E L I G K P K P Y Y
E I G E R V D Y K C K K G Y F Y I P P L
A T H T I C D R N H T W L P V S D D A C
Y R E T C P Y I R D P L N G Q A V P A N
G T Y E F G Y Q M H F I C N E G Y Y L I
G E E I L Y C E L K G S V A I W S G K P
P I C E K V L C T P P P K I K N G K H T
F S E V E V F E Y L D A V T Y S C D P A
P G P D P F S L I G E S T I Y C G D N S
V W S R A A P E C K V V K C R F P V V E
N G K Q I S G F G K K F Y Y K A T V M F
E C D K G F Y L D G S D T I V C D S N S
T W D P P V P K C L K V S H H H H H H

Fig. 13B

SEQ. ID NO:24

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CCCGAGGATACTGTAATAACGTACAAATGTGAAGAAAGCTTTGTGAAAATTCCTGGCGAGAAG
GACTCAGTGATCTGCCTTAAGGGCAGTCAATGGTCAGATATTGAAGAGTTCTGCAATCGTAGC
TGCGAGGTGCCAACAAGGCTAAATTCTGCATCCCTCAAACAGCCTTATATCACTCAGAATTAT
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GACCCTCAAGGGAATGGGGTTTGGAGCAGCCCTGCCCTCGCTGTGGAATTCTGGGTCAC
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GAATGCGATAAGGGTTTTTACCTCGATGGCAGCGACACAATTGTCTGTGACAGTAACAGT
ACTTGGGATCCCCAGTTCCAAAGTGTCTTAAA//GTGTG//CATCACCATCACCATCAC
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**WESTERN BLOT OF HYBRID PROTEINS
DAF-IgG4, DAF-CR1BB, and DAF-CR1B**

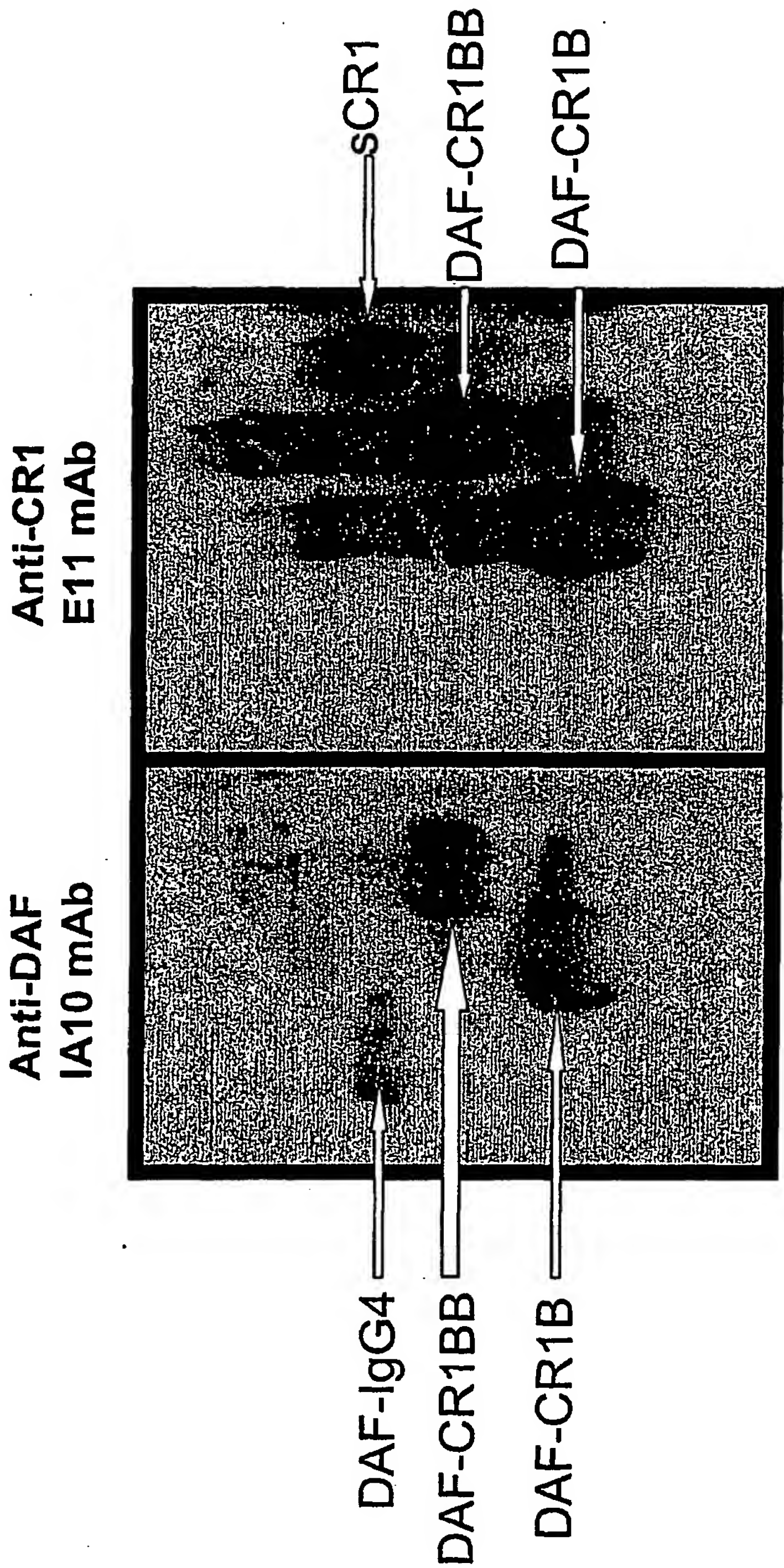


Fig. 14

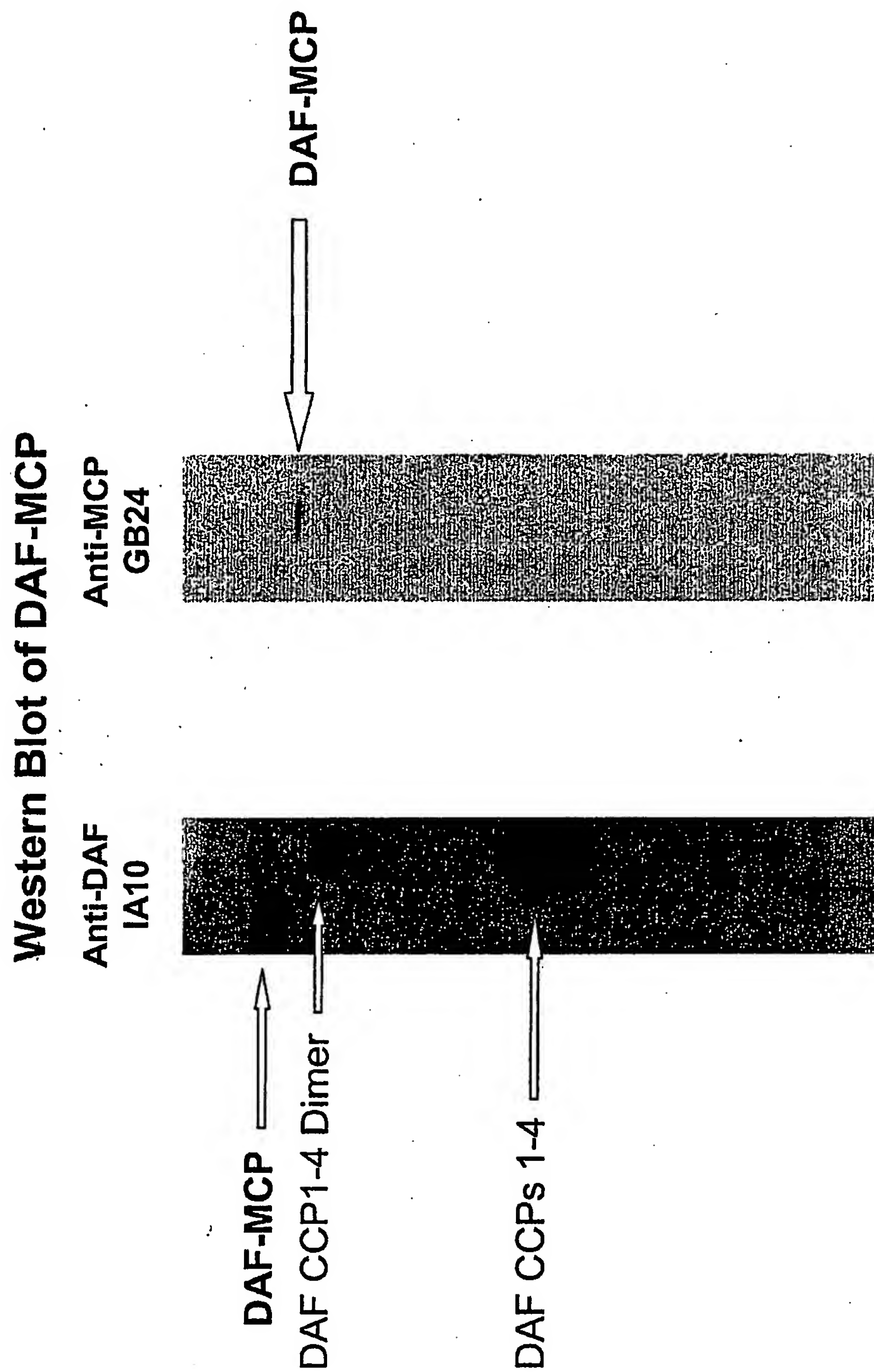


Fig. 15

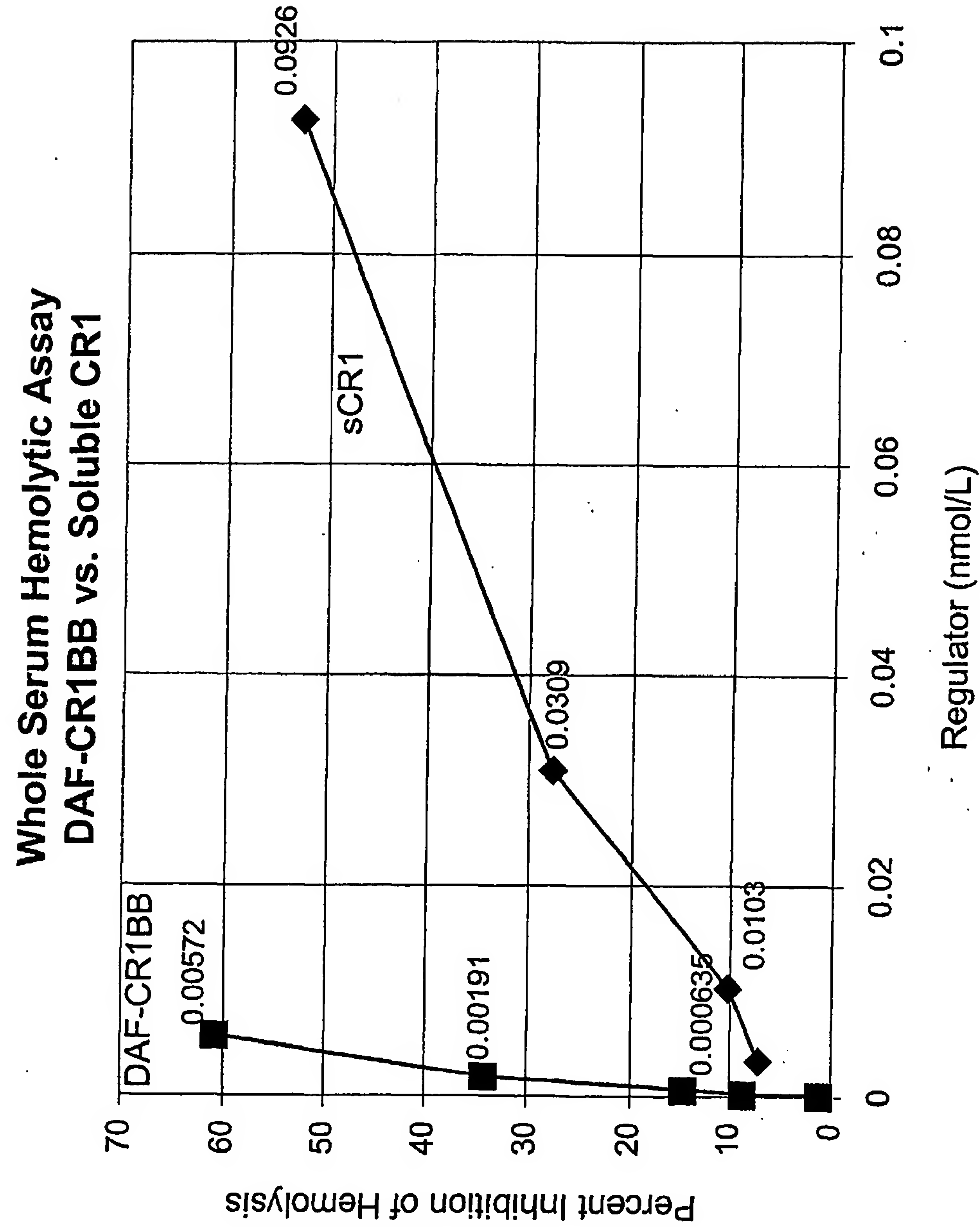


Fig. 16

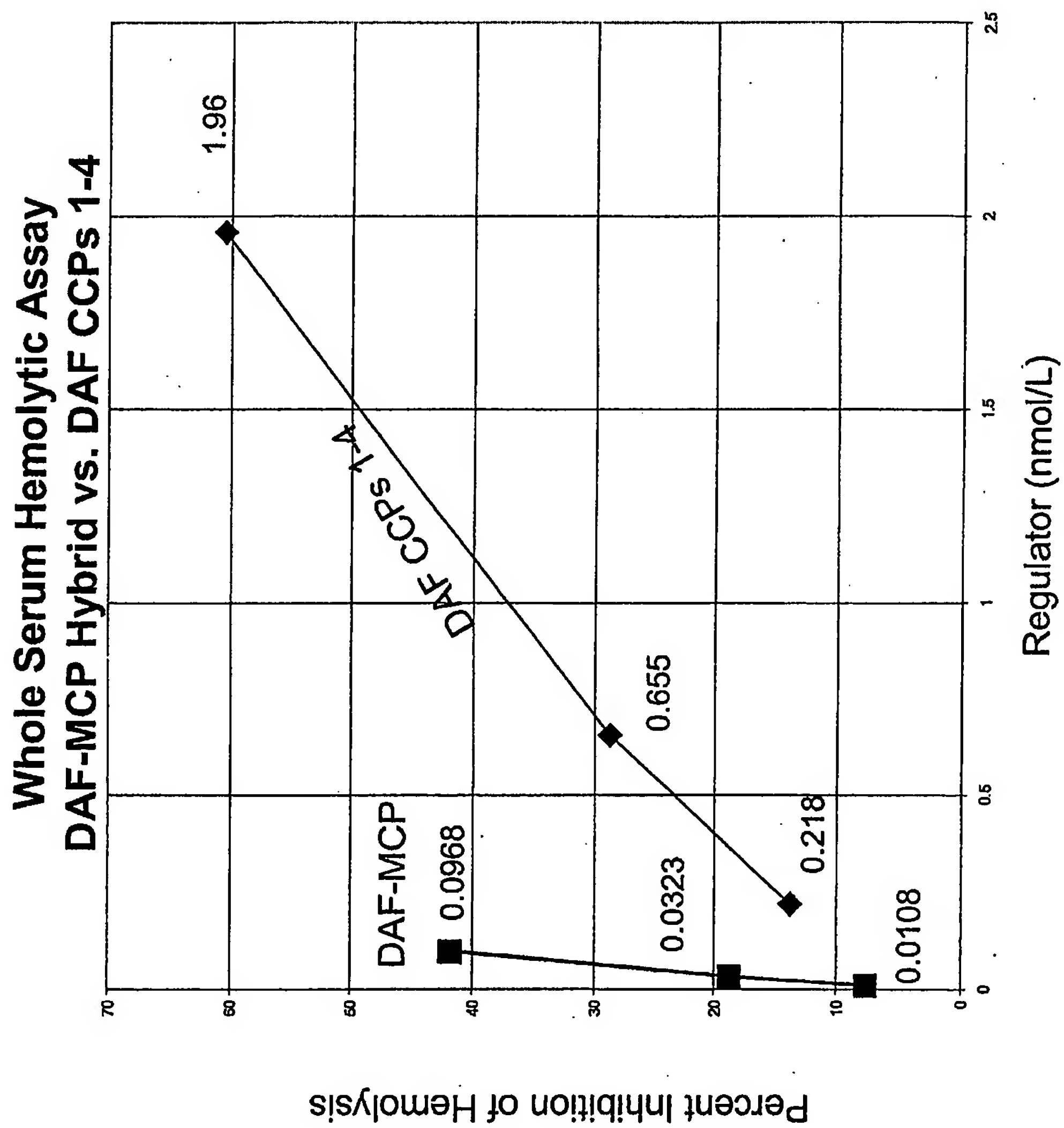


Fig. 17

Classical Pathway C3 Convertase Decay

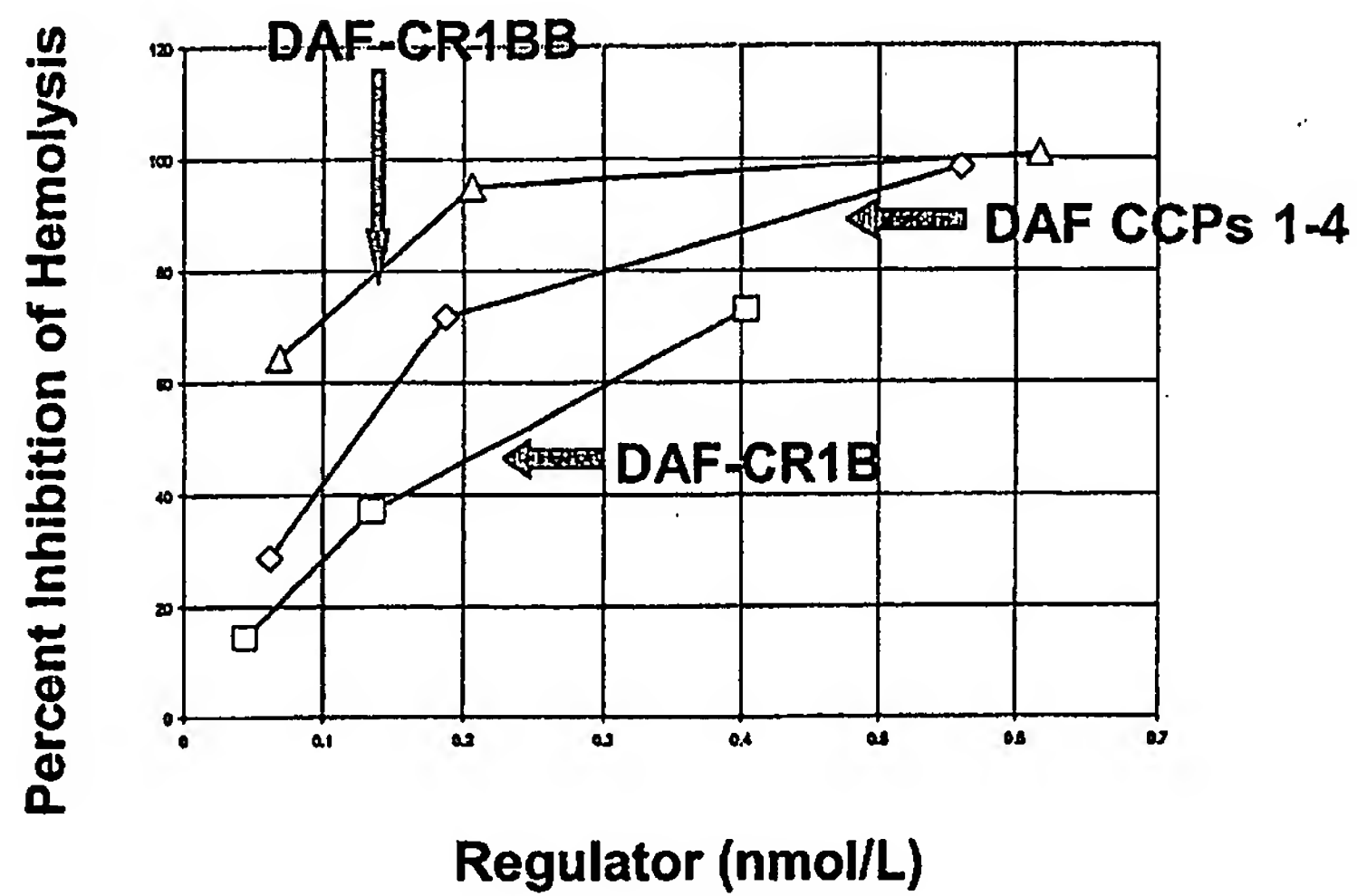


Fig. 18A

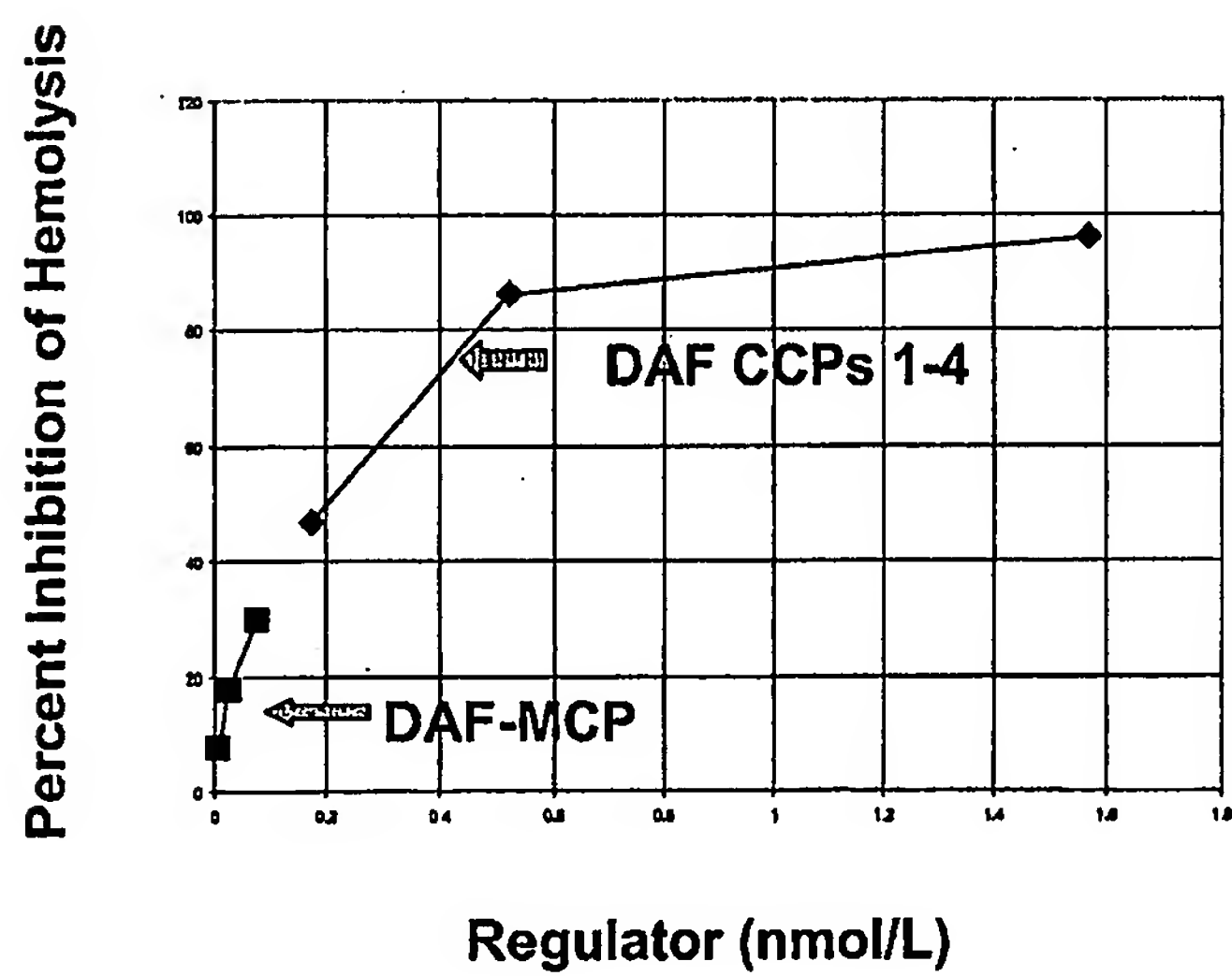


Fig. 18B

Classical Pathway C5 Convertase Decay DAF-CR1B vs DAF CCPs 1-4

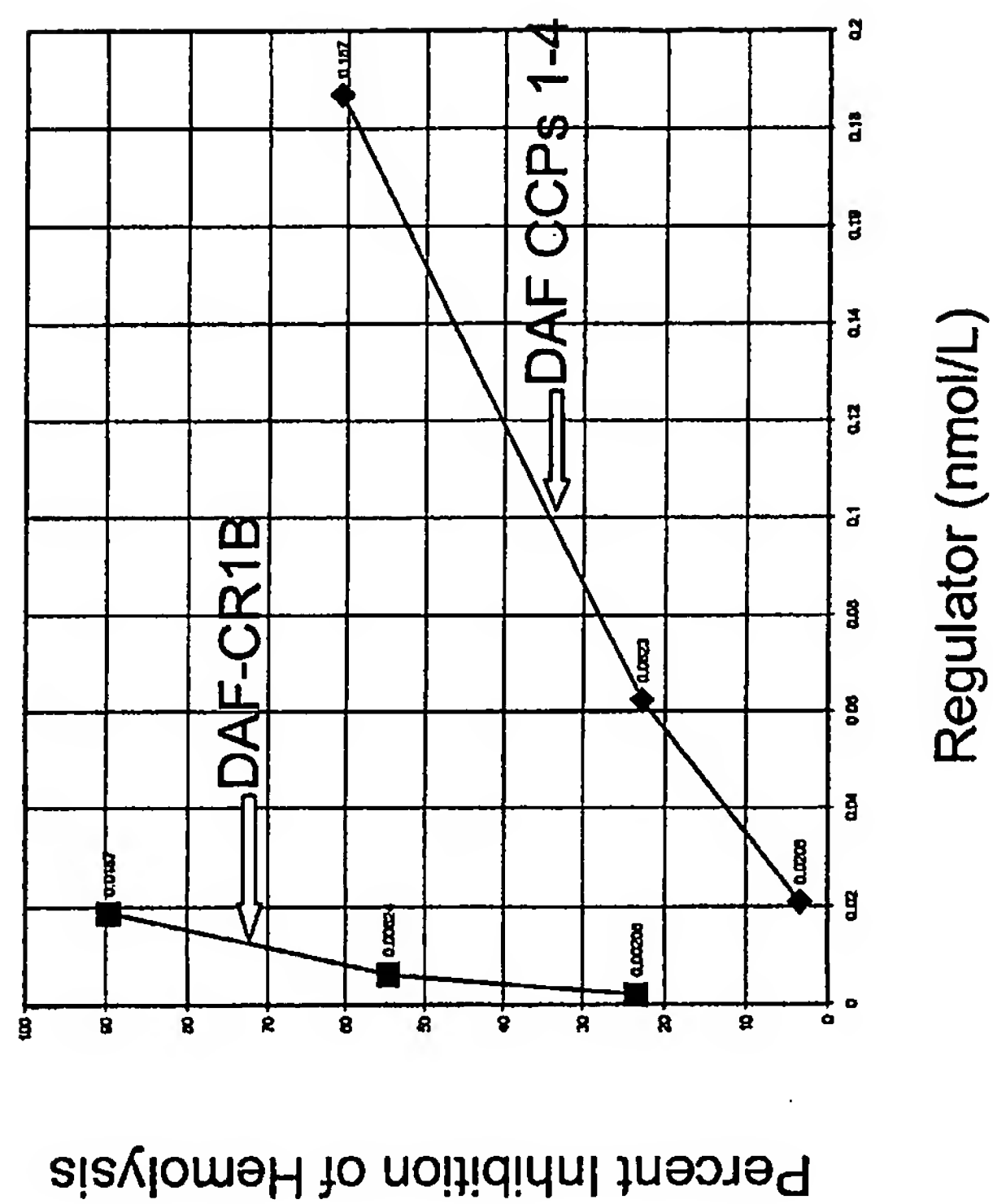


Fig. 19

Classical Pathway C5 Convertase Decay
DAF-CR1BB vs sCR1 vs DAF-CR1B

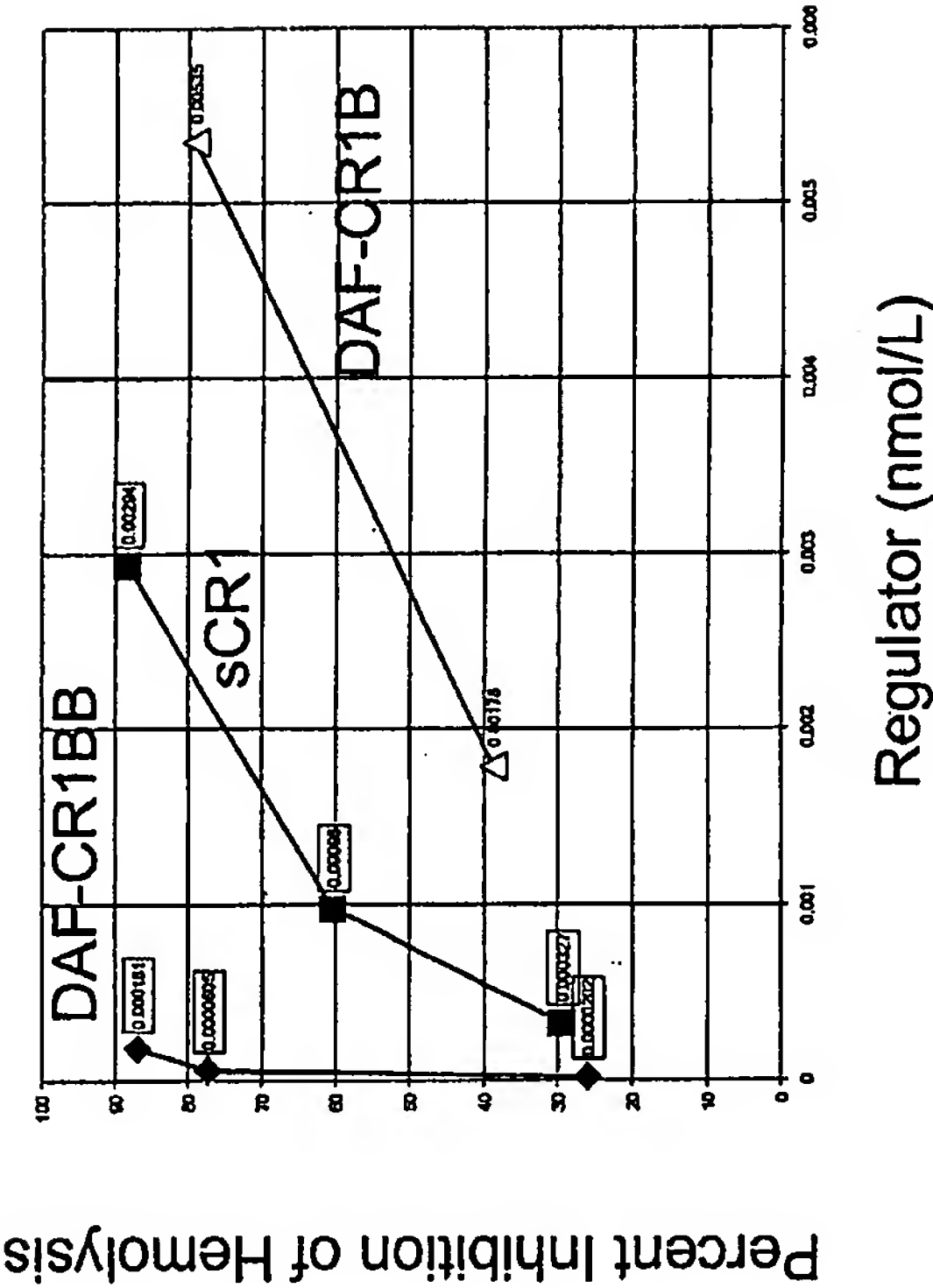


Fig. 20

Cell-bound (E^{sh}C4b3b) Cofactor Assay

Cell Supernatant

(Anti-human C3 pAb)

COS SN DAF-MCP DAF-CR1BB

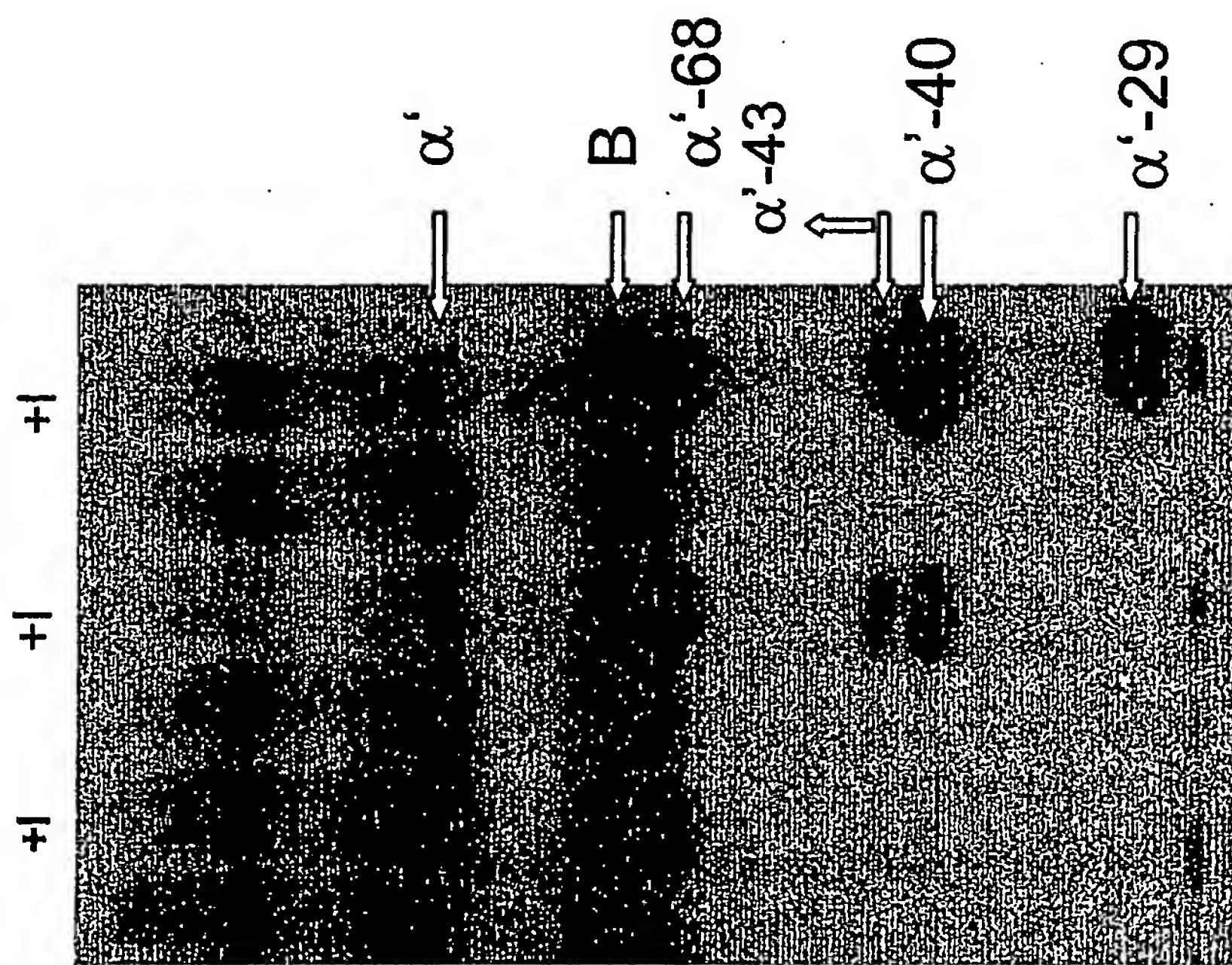


Fig. 21

Cell-bound (EshC4b3b) Cofactor Assays
Cell Supernatant

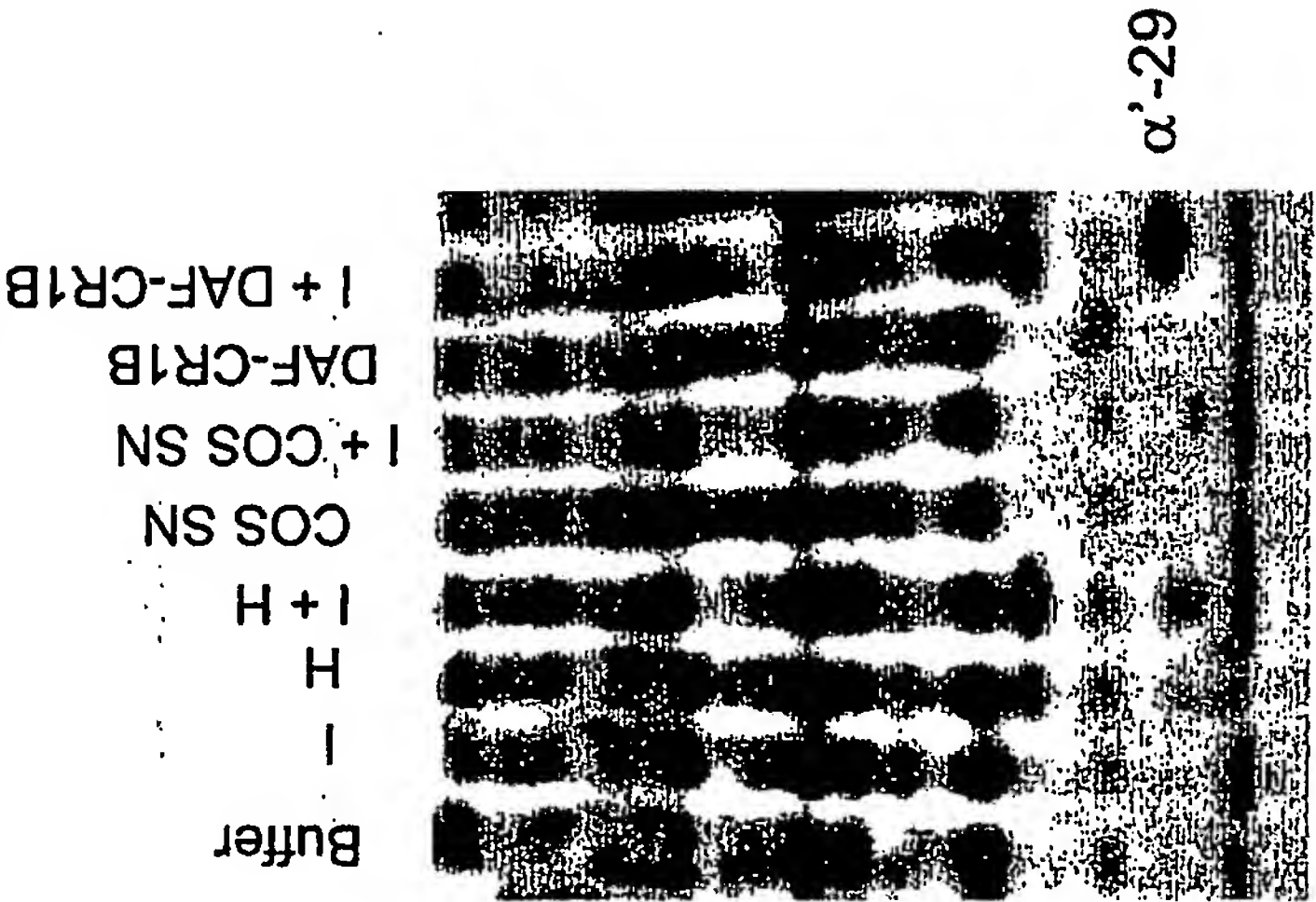


Fig. 22